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Impact of Insulin Resistance on Behavioral and Neurochemical Deficits in *db/db* Mice

A dissertation submitted in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

By
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Bachelor of Pharmacy, Amravati University, 2001
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2011
Wright State University

WRIGHT STATE UNIVERSITY
SCHOOL OF GRADUATE STUDIES

Date: November 02, 2011

I HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER MY SUPERVISION BY Ajaykumar Narayan Sharma ENTITLED Impact of Insulin Resistance on Behavioral and Neurochemical Deficits in *db/db* Mice BE ACCEPTED IN PARTIAL FUFILLMENT OF THE REQUIRMENTS FOR THE DEGREE OF Doctor of Philosophy.

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ABSTRACT

Ajaykumar Narayan Sharma (M.Pharm.), Biomedical Sciences Ph.D. Program, Wright State University, 2011. Impact of Insulin Resistance on Behavioral and Neurochemical Deficits in *db/db* Mice.

There is a high comorbidity of type-2 diabetes and neuropsychiatric disorders. However, there is paucity of preclinical research to study this phenomenon. The validity of the *db/db* mouse as an animal model to study type-2 diabetes and related macrovascular and microvascular complications is well established. The first part of this dissertation was designed to investigate comprehensively the *db/db* mouse behavior as preclinical evidence of type-2 diabetes related major neurobehavioral complications. Juvenile (5–6 weeks) and adult (10–11 weeks) *db/db* mice were screened for behavioral depression in forced swim test (FST), psychosis-like symptoms using pre-pulse inhibition (PPI) test, anxiety behavior employing elevated plus maze (EPM) test, locomotor behavior and thigmotaxis using open field test, emotional learning using fear potentiation of startle (FPS) test and working memory deficits in Y-maze test. Both juvenile and adult group *db/db* mice displayed behavioral despair with increased immobility time in FST. There was an age-dependent progression of psychosis-like symptoms with disrupted PPI in adult *db/db* mice. In the EPM test, *db/db* mice were less anxious as observed by increased percent open arms time and entries. They were also hypolocomotive as evident by a decrease in their basic and fine movements. There was no impairment of working memory in the Y-maze test in *db/db* mice. In the FPS test, *db/db* mice showed impaired learning response indicative of abnormalities in DA neuronal activity in the amygdala.

The second specific aim of this dissertation was to examine possible neurochemical basis for the observed behavioral deficits. Monoamine neurotransmitters dopamine (DA),

norepinephrine (NE) and serotonin (5-HT) are elemental to normal functioning of the brain. Current theories of the basis for major neurobehavioral disorders involve abnormalities in use of NE, 5-HT and DA in specific brain regions. Recent studies report that the DA agonist reverses diabetes-related metabolic abnormalities, thus suggesting that DA abnormalities may provide a link to underlying the psychiatric and endocrine disorders in type-2 diabetics. To evaluate type-2 diabetes related neurochemical alterations, concentrations of NE, 5-HT, DA and its catabolites homovanilic acid (HVA) and dihydroxyphenylacetic acid (DOPAC) were measured in the frontal cortex, amygdala, hippocampus, hypothalamus and brainstem of *db/db* mice by reverse-phase high performance liquid chromatography (HPLC). There was abnormal DA metabolism pattern in the frontal cortex and amygdala of *db/db* mice that correlated with their psychosis-like behavior and impaired FPS response. Moreover, type-2 diabetes related changes in the expression of DA biosynthesis rate determining step (RDS) enzyme tyrosine hydroxylase (TH) were studied using western blot technique. No changes in TH expression pattern were seen in the frontal cortex and amygdala of *db/db* mice compared to age-matched lean controls. Thus, abnormal DA metabolism pattern in these brain regions of *db/db* mice may be related to changes in the enzymatic activity of DA metabolizing enzymes and/or firing pattern of DAergic neurons.

The third specific aim of this research project was to examine the effect of long-term management of insulin resistance and hyperglycemia on neurobehavioral deficits in *db/db* mice. Rosiglitazone mixed in chow was administered to 5 week old *db/db* and lean control mice (20 mg/kg/day) for duration of 5 weeks. Separate groups of age-matched *db/db* and lean control mice were fed with standard chow. Mice were weekly monitored for blood glucose concentration. Five weeks after treatment onset, the mice were subjected to the forced swim test (FST), prepulse inhibition (PPI), open field test (OFT) and fear potentiated startle (FPS) test to examine for

behavioral depression, psychosis-like behavior, locomotor activity and emotional learning, respectively. Rosiglitazone-induced reversal of depression- but not psychosis-like behavior and impaired emotional learning in *db/db* mice.

The final specific aim of this project was to evaluate the effect of controlling insulin resistance and hyperglycemia on DA biosynthesis RDS enzyme TH, DA metabolism pattern and 5-HT and NE concentrations in selective brain regions of *db/db* diabetic mice. Rosiglitazone treatment resulted into restoration of the abnormal DA metabolism in the frontal cortex and amygdala of *db/db* mice to normal.

The principal findings of this dissertation report are: (1) *db/db* mice exhibit behavioral depression, age-dependent advancement of psychosis-like symptoms, impaired emotional learning and anxiolytic behavior; (2) abnormal DA metabolism pattern in the frontal cortex and amygdala of *db/db* mice that correlated with behavioral deficits; (3) rosiglitazone-induced reversal of depression- but not psychosis-like behavior and impaired emotional learning in *db/db* mice; and (4) restoration of the majority of changes observed in DA metabolism in the brains of *db/db* mice to normal post rosiglitazone treatment.

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(Ajaykumar Narayan Sharma)

1. Introduction

Diabetes mellitus is an endocrine metabolic disorder characterized by persistent hyperglycemia due to abnormalities in insulin secretion, insulin action or both. The occurrence of diabetes across the globe has grown at an alarming pace in the last decade. The World Health Organization (WHO) estimates that by the year 2030 around 370 million people worldwide will suffer with diabetes (Wild et al., 2004). Diabetes alone represents 11% of the total US healthcare expenditures (Hogan et al., 2003). During the last decade diabetes was sixth among fifteen of the most expensive health conditions in United States and second in terms of direct cost for individuals on medical treatment (Cohen and Krauss, 2003). Moreover, diabetes ranks sixth among major leading causes of death in the United States (Centers for Disease Control and Prevention).

1.1. Classification of diabetes

Diabetes can be divided into two main subtypes: (a) Type-1 diabetes was previously known as juvenile onset diabetes. It is an autoimmune disorder characterized by antibodies against insulin producing pancreatic β -cells resulting in insulin deficiency. β -cell specific antibodies appear long before the onset of type-1 diabetes. Approximately 10 percent of the diabetic population suffers from type-1 diabetes. (b) Type-2 diabetes was in the past referred to as mature onset diabetes. Type-2 diabetes accounts for around 90% of the diabetic population. It is a heterogeneous disorder which is generally the by-product of an interaction between environmental factors and genetic predisposition. There is reduced response to insulin by target tissues. Although blood insulin levels in type-2 diabetes patients are higher than type-1 diabetic patients, it is debatable to claim that type-2 diabetes is simply the outcome of insensitivity of

target organs to insulin. Though insulin levels are higher, they are not sufficiently high enough to overcome the insulin resistance observed in target organs like liver, muscle and adipose tissue. Thus, there is absolute insulin deficiency in type-1 diabetes while there is relative insulin deficiency in type-2 diabetes. Therefore, type-2 diabetes is currently considered to be a result of reduced insulin secretion plus target organ insensitivity to insulin.

1.2. Diabetes and co-morbid complications

Diabetes associated co-morbid complications can prove expensive as well as life threatening to diabetic patients. Individuals with co-morbid conditions account for 95% of inpatient hospitalizations for diabetes than diabetes alone. Diabetic patients are highly susceptible to cardiovascular system (CVS) disease, renal disease, neurological disorders, peripheral vascular disease, endocrine complications and retinopathies.

Diabetes related co-morbid central nervous system (CNS) complications

In addition to cardiovascular complications of diabetes, co-morbid neurobehavioral deficits create additional challenges to patients and healthcare practitioners. Many clinical reports have shown co-morbidity of type-2 diabetes with major neuropsychiatric disorders. Depression (Eaton et al., 1996; Kawakami et al., 1999; Musselman et al., 2003), schizophrenia (Cohen et al., 2006; Dickerson et al., 2008; Levitt Katz et al., 2005; Lin and Shuldiner, 2010), anxiety (Collins et al., 2009) and cognitive impairment (Gispén and Biessels, 2000) are some of the major complications associated with diabetes.

1.3. Diabetes and depression

Behavioral depression is the most common co-morbid disorder in the diabetic population (Katon, 2008). The odds of type-2 diabetic patients developing depressive symptoms are double

compared to the non-diabetic population (Anderson et al., 2001). Additionally, depressed patients are 37% more vulnerable to the onset of type-2 diabetes compared to non-depressed subjects (Knol et al., 2006). Further, among diabetic patients, individuals with co-morbid minor or major depression are at greater risk of mortality. Minor depression plus diabetes increases mortality 1.67 fold while major depression plus diabetes increases mortality 2.3 fold. Such depression associated increased mortality in diabetic patients may be linked to biological, neurochemical and behavioral factors. Behavioral despair in diabetic patients can lead to apathy towards self-care regimens such as regular exercise, dietary habits, cessation of smoking and towards medication for diabetes as well (Lin et al., 2004). Moreover, diabetic patients with co-morbid depression are reported to have doubled probability of cardiac risk factors like smoking, obesity, sedentary lifestyle etc (Katon et al., 2004). Interestingly, one meta-analysis study linked depressive symptoms in diabetic subjects to poor glucose regulation (Lustman et al., 2000).

1.4. Diabetes and schizophrenia

The co-morbidity of diabetes and schizophrenia has been acknowledged for more than 130 years. Sir Henry Maudsley famously quoted in his book '*The pathology of mind*' that '*Diabetes is a disease which often shows itself in families in which insanity prevails*' (Maudsley H, 1879). Peveler and Fairburn suggested that for the effective management of diabetes, treatment of co-morbid mental illness is necessary (Peveler and Fairburn, 1989). There is a 17-50% probability of developing schizophrenia in the type-2 diabetic population or in people with a family history of type-2 diabetes (Mukherjee et al., 1989). Moreover, a higher incidence (~20 percent) of schizophrenia, attention deficit hyperactivity disorder (ADHD), bipolar disorder, depression and neurodevelopmental disorder was reported at the onset of type-2 diabetes in children (Levitt Katz

et al., 2005). In the year 2003, a group of diabetologists and psychiatrists discussed the co-morbidity of diabetes and schizophrenia and developed guidelines to deal with their association (Holt, 2004). Co-morbid type-2 diabetes is reported to impair physical as well as mental health status in adult schizophrenic patients compared to non-diabetic schizophrenics (Dickerson et al., 2008; Dickinson et al., 2008). The pioneer work showing an association between the use of typical antipsychotic medications and the development of type-2 diabetes was made in 1956 (Hiles, 1956). This led to the introduction of the term '*phenothiazine diabetes*'. The emergence of new-onset diabetes was also reported in schizophrenic patients after initiation of atypical antipsychotic medications (Lambert et al., 2006; Ramaswamy et al., 2006; van Winkel et al., 2008). Hyperglycemia and insulin resistance was also reported in mice that received chronic antipsychotic treatment (Dwyer and Donohoe, 2003). Recently van Nimwegen and co-workers showed that antipsychotic-naïve schizophrenics also experience insulin resistance compared to non-schizophrenic controls. Such increased propensity towards insulin resistance in schizophrenics even when not medicated may be the outcome of metabolic abnormalities (van Nimwegen et al., 2008). These findings hint toward a bidirectional link between diabetes and schizophrenia. Surprisingly, there are no preclinical attempts to examine the development of psychosis-like behavior in animal models for type-2 diabetes. Despite all the recent interest in the potential link between diabetes and schizophrenia, the underlying mechanisms for their co-morbidity remains poorly understood.

1.5. Diabetes and anxiety

A comprehensive meta-analysis of scientific literature suggest that anxiety disorders are directly linked with diabetes related hyperglycemia (Anderson et al., 2001). A recent large-scale population based meta-analysis study (n = 37,291) suggested anxiety as a risk factor for

development of type-2 diabetes (Engum, 2007). Further, high anxiety scores (Collins et al., 2009) and an increased prevalence of generalized anxiety disorder were reported in type-2 diabetic patients relative to non-diabetic adults (Fisher et al., 2008). For most of type-2 diabetic patients, emotional problems such as anxiety remain largely undetected (Pouwer, 2009). Because of the lack of awareness of this co-morbidity there is a dearth of intervention studies to treat such co-morbid symptoms (Pouwer, 2009). Alternatively, Labad and colleagues recently have shown that depression but not anxiety is associated with type-2 diabetes (Labad et al., 2010). Thus, clinical studies evaluating anxiety levels in type-2 diabetic patients led to conflicting outcomes. There are few preclinical reports that attempted to study anxiety behavior in animal models of diabetes. In spontaneously diabetic *INS2^{Akita}* mouse, Asakawa and colleagues reported anxiety-like behavior using the elevated plus maze test (Asakawa et al., 2007). Further, type-2 diabetic *ob/ob* mice were reported to exhibit anxiety behavior (Asakawa et al., 2007; Finger et al., 2010). Exploring the biological mechanisms that govern anxiety-like behavior using mouse model for type-2 diabetes may help to improve our understanding about their co-morbidity.

1.6. Diabetes and cognition

Cognitive deficits are found in type-2 diabetic mice (Li et al., 2002) and are also prevalent in type-2 diabetic patients (Gispen and Biessels, 2000). *db/db* mice have impaired spatial memory task performance in the Morris water maze test (Li et al., 2002). While the frontal cortex is the anatomical site for working memory, the hippocampus's role as the substrate for spatial memory functions is well documented (Bohlen und et al., 2006; Courtney et al., 1998). Recently, Stranahan and colleagues reported diabetes-induced detrimental effects on hippocampal neurons, impaired recovery from hippocampal neuronal defects post-calorie restriction and increased

energy expenditure in insulin-resistant *db/db* mice (Stranahan et al., 2009). Elevations in corticosterone levels (Stranahan et al., 2008) as well as reduced hippocampal brain-derived neurotrophic factor (BDNF) levels (Stranahan et al., 2009) are suggested as crucial mechanisms for diabetes-related cognitive deficits. Ohta and colleagues using a conditioned taste aversion (CTA) learning test found that impaired downstream signaling due to the mutation in leptin receptors in *db/db* mice had no effect on acquisition of CTA learning but promoted faster extinction (Ohta et al., 2003). Yamamoto suggested that the parabrachial nucleus, amygdala, insular cortex, supramammillary nucleus, nucleus accumbens, and ventral pallidum as possible anatomical sites for CTA learning in rats (Yamamoto, 2007).

1.7. Diabetes and stress

Emotional stress has long been considered as a risk factor for the development of diabetes. The English physician Thomas Willis commented that diabetes is more common in people that had experienced emotional stress (Willis, 1675). Stress may be an important contributing factor to the development of diabetes and co-morbid disorders (Castaneda et al., 2011; Trento et al., 2010). Recently in a mouse model of post-traumatic stress disorder (PTSD), Castaneda and co-workers reported impaired glucose tolerance (Castaneda et al., 2011). Measurement of glycated hemoglobin (HbA_{1C}) is a laboratory test used to determine average blood glucose concentration over a prolonged time. According to the American Diabetes Association, HbA_{1C} $\geq 6.5\%$ is the confirmatory test for diagnosis of diabetes. In a cross-sectional study there was a strong association between lifetime PTSD symptoms in low-income patients and $> 7\%$ HbA_{1C} levels (Miller et al., 2011). Alternatively, PTSD is a risk factor for the development of diabetes (Weiss et al., 2011).

1.8. Impact of insulin resistance and hyperglycemia on brain functions

Multiple clinical studies suggest a close relationship between insulin resistance, hyperglycemia and neuropsychiatric disorders (Anderson et al., 2001; Cohen et al., 2006; Collins et al., 2009; de la Monte et al., 2009; Gispen and Biessels, 2000; Hall et al., 2009; Koopmans et al., 2009; Pouwer, 2009). Peripheral insulin resistance correlates with insulin insensitivity in the brain and increases the vulnerability of neurons to degeneration. In streptozotocin-induced diabetic rats, insulin treatment normalizes hyperglycemia-induced deficits in nerve conduction velocity (Huang et al., 2003). Several studies suggested a regulatory role for insulin in brain glucose metabolism, neurobehavioral functions and inflammatory responses in the CNS. Diabetes associated insulin resistance and hyperglycemia affects neuronal morphology (Malone et al., 2008) and severely damages the brain (Jacob et al., 2002). Hyperglycemia amplifies brain levels of oxidative stress markers and induces inflammation (Tsuruta et al., 2010). Such close integration between insulin resistance and brain functions suggests that improving insulin sensitivity and normalization of hyperglycemia may help to overcome some of the damage and neurobehavioral outcomes. Rosiglitazone, a selective agonist at peroxisome proliferator activated gamma (PPAR γ) receptors improves insulin-sensitivity of target organs. Rosiglitazone also lowers the severity of depression in insulin-resistant depressed patients (Rasgon et al., 2010). Moreover, rosiglitazone in type-2 diabetics can improve working memory performance (Ryan et al., 2006). The PPAR γ receptor agonist pioglitazone produced antidepressant-like effects in non-diabetic mice, while administration of a PPAR γ antagonist prior to pioglitazone attenuated this effect (Sadaghiani et al., 2011). These results suggest that PPAR γ receptor activation in the brain contributes to some of the beneficial effects of insulin sensitizing drugs independently of its

effect on blood glucose. Further, a neuron-specific PPAR γ receptor knockout in high-fat diet mice decreased rosiglitazone-induced improvement in glucose metabolism (Lu et al., 2011) suggesting a role of central PPAR γ receptors in hepatic insulin sensitivity.

1.9. Dopamine as a novel factor in type-2 diabetes complications

There is a rapidly growing interest in the involvement of DA in the pathophysiology of type-2 diabetes. Administration of a DA neurotoxin into the brain induces insulin resistance (Luo et al., 1997; Pijl, 2003). There is decreased DA neurotransmission and DA receptor sensitivity in rodent models for diabetes (Cincotta et al., 1997; Rutledge et al., 2002). Type-2 diabetic Zucker *fa/fa* rats have diminished D₂R expression in the hypothalamus (Fetissov et al., 2002), decreased DA levels, and impaired post-synaptic DA action (Meguid et al., 2000). Type-2 diabetic *ob/ob* mice have diminished locomotive response to amphetamine (Fulton et al., 2006). Further, D₂R knockout mice have an impaired insulin response to glucose, high fasting glucose and glucose intolerance (Garcia-Tornadu et al., 2010a). Interestingly, 4-week rosiglitazone treatment (10 mg/kg) to Zucker *fa/fa* rats has been shown to restore renal D₁R number and dopamine mediated urinary sodium excretion (Umrani et al., 2002). Clinical and preclinical reports suggest region-specific alterations in DA and its catabolites in the diabetic brain (Lackovic et al., 1990).

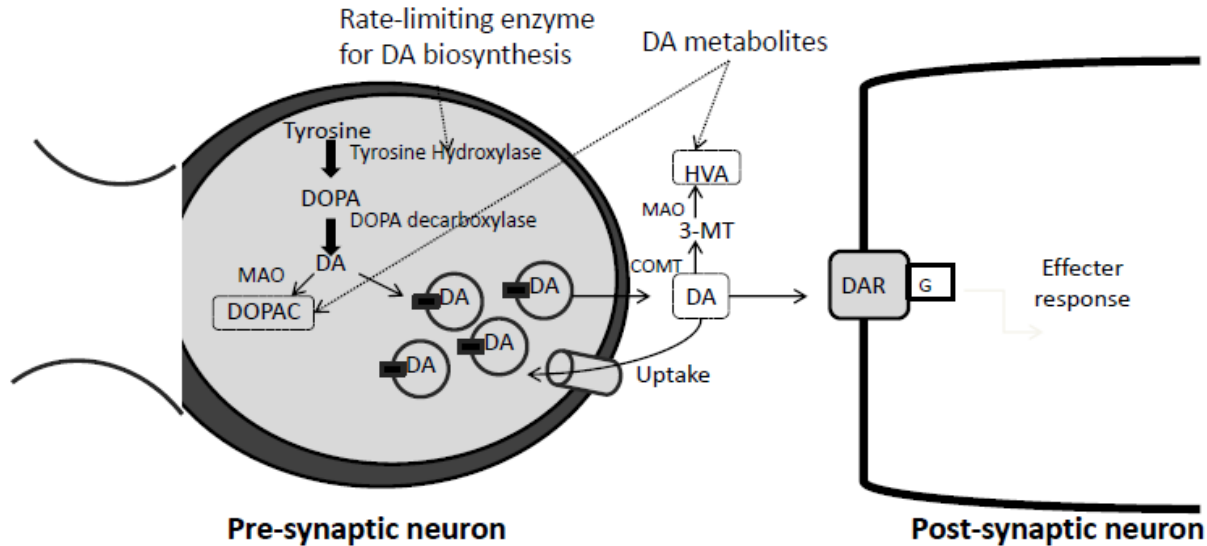
Interestingly, activation of D₂Rs in the *ob/ob* mice helped to lower diabetes-related metabolic abnormalities (Cincotta et al., 1997). Recently, the FDA approved the use of the D₂R agonist, bromocriptine, for the treatment of type-2 diabetes (de Leeuw van Weenen JE et al., 2010; Gaziano et al., 2010; Scranton and Cincotta, 2010). D₂R agonist can normalize hyperglycemia and glucose-stimulated insulin secretion (de Leeuw van Weenen JE et al., 2010; Liang et al., 1998; Scislowski et al., 1999). D₂R agonist also augments the antidepressant effect of selective

serotonin reuptake inhibitors (Renard et al., 2001) and is well known to stimulate motor behavior (Bruhwyler et al., 1991).

Tyrosine Hydroxylase

Tyrosine hydroxylase (TH) is the rate-limiting enzyme for the biosynthesis of DA. In addition to changes in DA neurotransmission, receptor sensitivity and metabolite concentrations, a fall in TH levels was reported in *ob/ob* diabetic mice brain (Fulton et al., 2006). There are few studies that attempted to study diabetes-induced changes in brain TH activity. Type-1 diabetic rats show increased TH expression in the cerebral hemispheres, cerebellum, brainstem and olfactory lobes (Gupta et al., 1992). Further, TH expression was increased in the locus coeruleus but decreased in the ventral tegmental area (VTA) and substantia nigra of type-1 diabetic rats (Figlewicz et al., 1996). Also, structural deformities in nerve fibers containing TH were reported in type-1 diabetic rats (Tsai et al., 2008). Few laboratories have looked for type-2 diabetes related changes in TH in only selected brain regions. A fall in TH concentration was reported in the nucleus accumbens (NAc) and VTA of leptin-deficient *ob/ob* mouse brain (Fulton et al., 2006). However, TH immunoreactivity in neurons of Zucker *fa/fa* rats were not different from that of lean control rats (Fetissov et al., 1997). Thus, changes in TH expression in type-2 diabetic brain may be region-specific or model specific. Evaluation of TH concentrations in different brain regions of *db/db* mice could help us to understand role of this enzyme in progression of type-2 diabetes related behavioral and neurochemical deficits.

1.10. Dopamine in the brain



Dopamine biosynthesis and metabolism

Abbreviations: DOPA – Dihydroxyphenylalanine; DA – Dopamine; MAO – Monoamine Oxidase; DOPAC – Dihydroxyphenylacetic Acid; COMT – Catechol-O-Methyl Transferase; 3-MT – 3-Methyltyrosine; HVA – Homovanillic Acid; DAR – Dopamine Receptor; G – G-Protein.

DA is a key neurobiological substrate involved in schizophrenia (Moncrieff, 2009; Remington, 2008) and depression (Butler and Meegan, 2008; Guiard et al., 2009). A decline in cerebrospinal fluid HVA concentration, a major DA metabolite, was reported in suicide attempters and depressed patients relative to controls (Engstrom et al., 1999; Volkow et al., 2000). However, the DA concentrations in the brains of suicidal victims remain unaffected suggesting reduced DA metabolism (Bowden et al., 1997). Antidepressant treatment increases D₂R density in the rat brain (Dziedzicka-Wasylewska et al., 1997). Further, schizophrenics have reduced DA concentrations and TH immunoreactivity in the dorsolateral prefrontal cortex (Akil et al., 2000;

Weinberger et al., 1988). The brain regions that we planned to study for changes in DA metabolism all receive dopaminergic neuronal projections (Bjorklund et al., 1975; Lazar et al., 2008; Mehler-Wex et al., 2006; Nakasato et al., 2008; Stevenson and Gratton, 2004; Villablanca, 2010). DA in the frontal cortex (FC) regulates some behaviors such as pre-pulse inhibition of startle (PPI) (Lazar et al., 2008; Mehler-Wex et al., 2006; Nakasato et al., 2008). DA hypoactivity in the FC induces psychosis-like behavior as well as impairment of pre-pulse inhibition of startle (Bacopoulos et al., 1979; Davis et al., 1991). The amygdala, a component of the mesolimbic DA system, participates in conditioned fear (Fadok et al., 2009) and fear potentiation of startle (FPS) (Stevenson and Gratton, 2004) behaviors. Caudate DA coordinates voluntary movements (Villablanca, 2010) and DA hypoactivity in the caudate can promote hypo-locomotion. The hippocampus is a primary component of the mesolimbic dopaminergic system involved in cognitive functions and dopaminergic projections to the hypothalamus regulates neuroendocrine functions (Bjorklund et al., 1975).

Dopamine pathways in the brain

DA pathway	Cell bodies	Neuronal projection	Physiological role	Pathophysiology
Mesocortical (A10)	Ventral Tegmental Area (VTA)	Neocortex (Frontal cortex)	Concentration, working memory	Psychosis (DA hypo-frontality)
Mesolimbic (A10)	VTA	Limbic system (Amygdala, Hippocampus)	Motivation, reward.	Psychosis (DA hyperactivity) Depression (↓ CSF HVA)
Nigrostriatal (A9)	Substantia Nigra (SN)	Dorsal striatum (Caudate)	Coordination of voluntary movements	Parkinsonism (Hypolocomotion) EPS/Catalepsy [(−) D ₂ -R]
Tubero-infundibular	Hypothalamus (Arcuate, PVN)	Median eminence, intermediate lobe	(−) prolactin release (Ant. Lobe)	Hyperprolactinemia

1.11. Dopamine and glucose metabolism

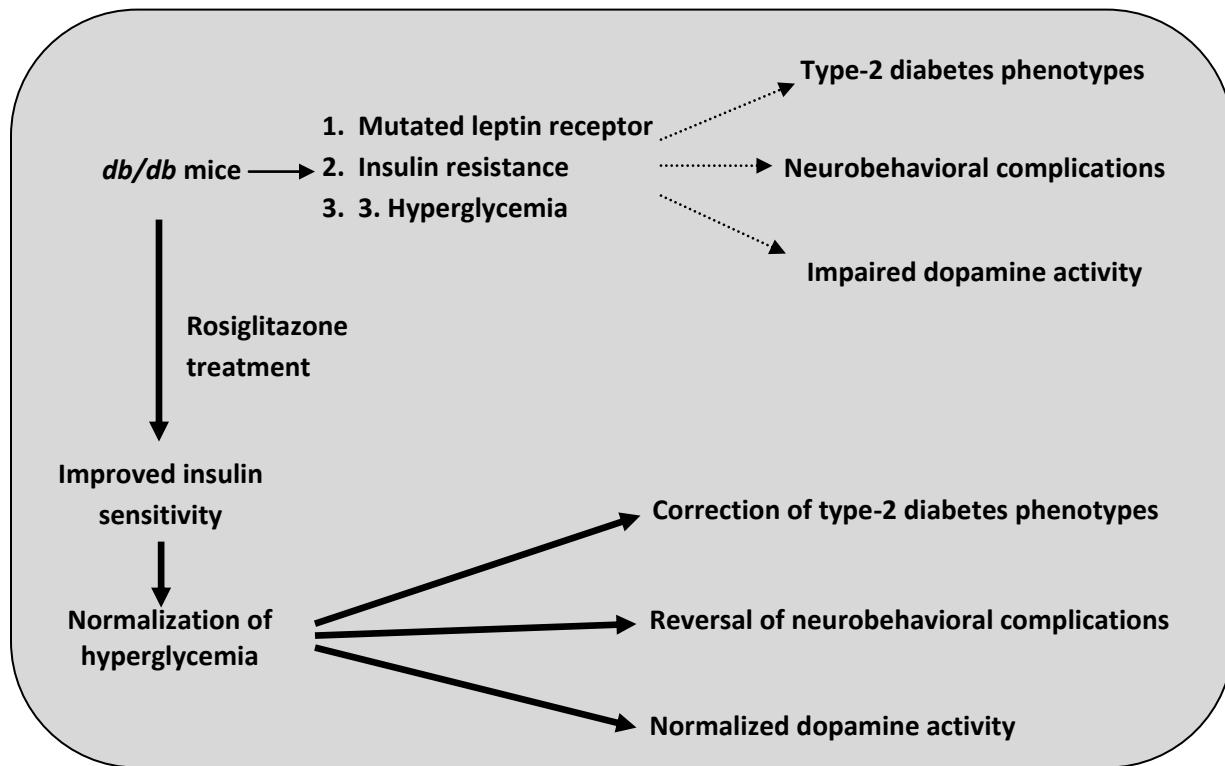
Changes in DA neurotransmission have been shown to have a great impact on glucose metabolism (Cincotta and Meier, 1996; Cincotta et al., 1997). Insulin resistant animal models have reduced DA neurotransmission in the brain (Lemierre et al., 1998; Meguid et al., 2000; Orosco et al., 1995) and decreased DA receptor expression in the hypothalamus and striatum (Fetissov et al., 2002; Pijl, 2003; Wang et al., 2001). DA neurotoxins induce insulin resistance (Luo et al., 1997). Insulin resistant *ob/ob* mice have reduced DOPAC/DA ratios in the frontal cortex and brainstem and increased HVA/DA ratios in the frontal cortex and hypothalamus (Harris et al., 1998). A clinical report also suggests region-specific alterations in DA and its catabolites in the brains of diabetic patients (Lackovic et al., 1990). Interestingly, activation of D₂Rs in the insulin resistant *ob/ob* mice helped to lower diabetes-related metabolic abnormalities (Cincotta et al., 1997). Further, the majority of current antipsychotic medications that are D₂R antagonists promote hyperglycemia, insulin-resistance and weight gain, which worsens pre-existing diabetes and related complications (De Hert and Cohen, 2009; Dwyer et al., 2001; Dwyer and Donohoe, 2003; Lipscombe et al., 2009). Thus, insulin resistance and hyperglycemia could impair DA biosynthesis and/or metabolism in the selective brain regions of *db/db* mice. Approaches to improve insulin sensitivity may help to overcome some of these type-2 diabetes dopaminergic abnormalities.

2. Development of hypothesis

A substantial body of research points to an increased risk of brain disorders such as depression and schizophrenia in type-2 diabetic patients. However, the underlying mechanisms for such co-morbidity remain poorly understood. Insulin resistance is the hallmark feature of type-2 diabetes. Insulin sensitizers such as rosiglitazone lower insulin resistance and hyperglycemia in type-2 diabetic patients. In addition to their antidiabetic effect, insulin sensitizers are shown to reverse depression and improve working memory performance. Compelling evidences links type-2 diabetes to impairments in dopamine (DA) signaling. DA receptor agonists normalize hyperglycemia and insulin secretion. Further, DA receptor antagonists augment insulin resistance and hyperglycemia. DA also plays a critical role in the pathophysiology of schizophrenia and depression. The co-localization of insulin and DA receptors in several brain regions opens the possibility of insulin regulation of DA neurotransmission in the diabetic brain. We hypothesize that insulin resistance impairs DA biosynthesis and metabolism in selective brain regions and this could contribute to type-2 diabetes related neurobehavioral deficits. Improvement of insulin sensitivity and control of hyperglycemia may reverse type-2 diabetes related selective behavioral and neurochemical deficits. The *db/db* mouse is an animal model for type-2 diabetes which has the characteristic insulin resistance and hyperglycemia.

The central hypothesis is: Type-2 diabetic *db/db* mice experience behavioral and neurochemical (dopaminergic) deficits. Effective management of insulin resistance and related hyperglycemia will significantly reverse some of behavioral and neurochemical deficits in *db/db* mice.

Hypothesized mechanism



3. Specific aims

Specific aim 1: To test the hypothesis that type-2 diabetic *db/db* mice have neurobehavioral deficits.

db/db mice were used as a model to evaluate behavioral and diabetic phenotypes. Mice were screened for depression, psychosis, anxiety, locomotion, working memory and emotional learning. A battery of six behavioral tests was used to meet this objective.

Specific aim 2: To test the hypothesis that *db/db* mice have altered DA biosynthesis and/or metabolism in selective brain regions.

Brain regions such as the frontal cortex, amygdala, hippocampus, hypothalamus and brainstem were evaluated for DA biosynthesis and metabolism patterns. Reverse-phase high performance liquid chromatography (RP-HPLC) was used to measure concentrations of DA and its metabolites HVA and DOPAC in selected brain regions with DA neuronal projections. Western-blots were used to examine type-2 diabetes related changes in DA biosynthesis RDS enzyme TH.

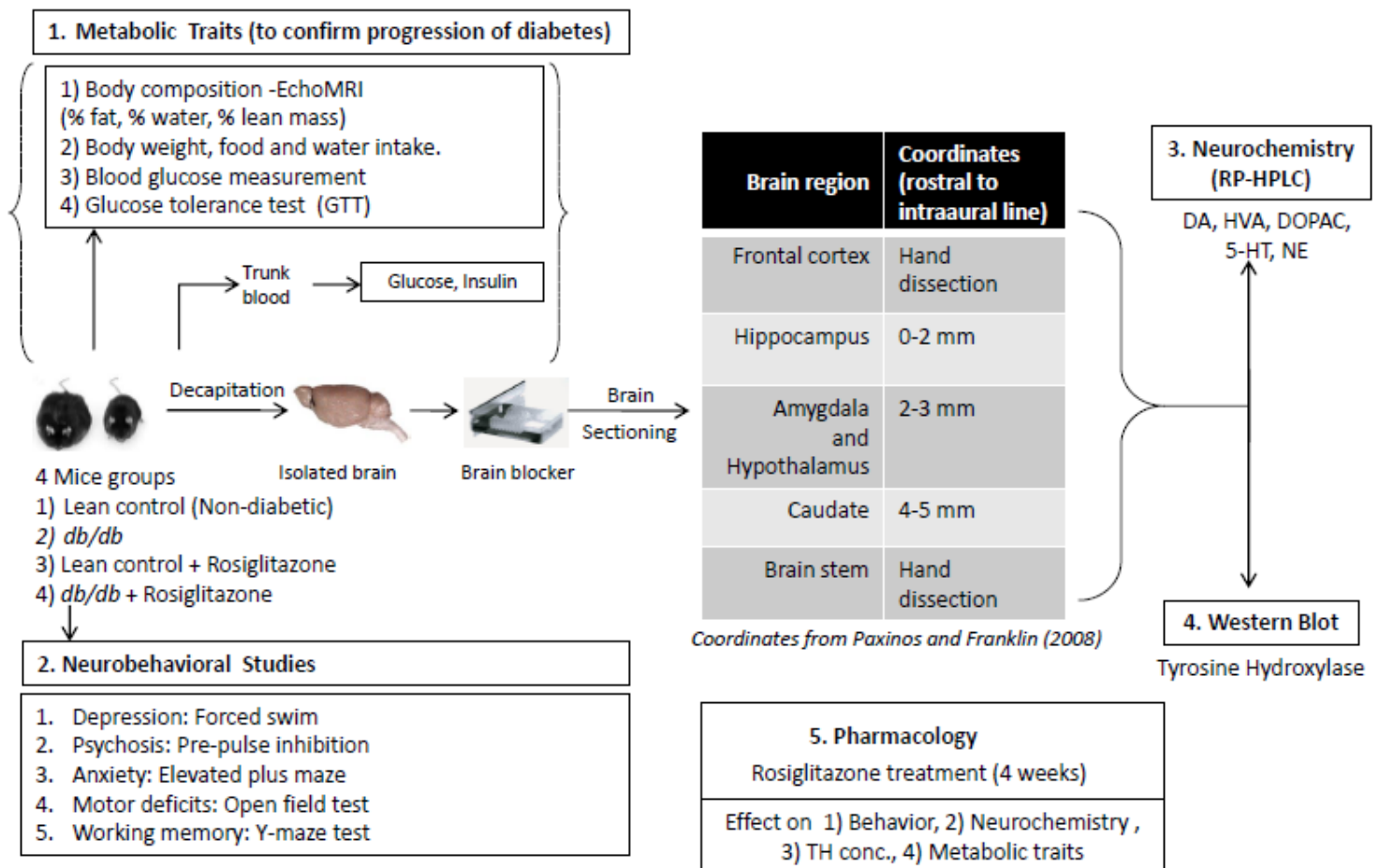
Specific aim 3: To test the hypothesis that improving insulin sensitivity and effective management of hyperglycemia will reverse some of neurobehavioral deficits in *db/db* mice.

db/db mice were fed with rosiglitazone to improve insulin sensitivity and blood glucose concentrations. Selective behavioral tests were run based on specific aim 1 experimental results to study the impact of improving insulin sensitivity and normalization of hyperglycemia on neurobehavioral deficits in *db/db* mice.

Specific aim 4: To test the hypothesis that improving insulin sensitivity and effective management of hyperglycemia will reverse alterations in DA biosynthesis and/or metabolism in

the *db/db* mouse brain. The effect of improving insulin sensitivity on DA biosynthesis and metabolism pattern was studied in selective brain regions with DA neuronal projections.

3.1. Overall experimental plan



4. Materials and methods

4.1. Animals

Male *db/db* [5 week old; background strain C57BL/KsJ (BKS-Cg-Dock7^m *+/+* Lepr^{*db*}/J)] and their age-matched non-diabetic lean control mice were procured from Jackson Laboratories (Bar Harbor, ME). All the mice were singly housed in plastic cages with wooden shavings in a temperature controlled room (22–23 °C) with 12:12 h light : dark cycle (lights off at 1700). Principles of laboratory animal care were followed and all experimental protocols were approved by the Wright State University Animal Care and Use Committee.

Metabolic parameters

To assess the metabolic status as an index of progression of diabetes, body weights, food consumption and water intake of *db/db* and lean control mice was monitored weekly. Blood glucose (fed), plasma insulin concentrations and body fat composition were measured to verify development of diabetes.

4.2. Whole-body fat and lean mass measurements

The EchoMRI whole body composition analyzer (Houston, TX, USA) was used to determine fat and lean body mass of lean control and *db/db* mice (Taicher et al., 2003). The EchoMRI is a QNMR instrument that offers rapid measurement of whole-body composition parameters like total body fat, lean mass, body fluids and total body water in live mice without sedation and anesthesia. Briefly, EchoMRI instrument was calibrated followed by live mouse placement into a plastic cylinder (inside diameter: 4.7 cm; thickness: 0.15 cm). A plastic plunger was used to restrain mouse movements except to turn back and about 4 cm vertical movements. Fat and lean mass was calculated as percent of total mass.

4.3. Plasma glucose and insulin measurements

For blood glucose measurements, blood samples were taken from a cut made on the tip of the tail and glucose concentration was determined using an Accu-Check Advantage[®] (Roche Diagnostic Corporation, Indianapolis) or Free Style Lite[®] (Abott Diabetes Care, CA) Blood Glucose Monitor. Plasma glucose as well as insulin concentrations were also measured in 10 week old *db/db* mice and their age-matched lean controls that were not fasted. Mice were decapitated, and trunk blood was collected in ice-chilled heparinized tubes. Plasma was immediately separated and stored at -80°C . The plasma samples were analyzed at Mouse Metabolic Phenotyping Center (Cincinnati, OH) for insulin and glucose according to the manufacturer's specifications (Millipore, St. Charles, MO).

4.4. Glucose tolerance test (GTT)

To study glucose handling in mice, intra-peritoneal GTT was performed. Mice were fasted for 16 hours. Fasting blood glucose was measured after the end of fasting period. Mice were then injected with glucose (1.5 mg/kg, ip). Blood samples were taken from mouse tail vein at 15, 30, 45, 60, 90 and 120 minutes after intraperitoneal glucose injection. These blood samples were analyzed for glucose concentration by colorimetric assay using Glucose (GO) Assay Kit according to the manufacturer's specifications (Sigma, Missouri).

4.5. Behavioral studies

After 1 week acclimatization, mice were subjected to behavioral testing. At two different age points (juvenile: 5–6 weeks and adult: 10–11 weeks) separate groups of mice were subjected to 1) forced swim test, 2) pre-pulse inhibition test, 3) elevated plus maze test, 4) open field test, 5)

fear potentiated startle test and 6) Y-maze test. All the tests were conducted between the hours of 0900–1400 to minimize circadian influences on mouse behavior.

4.5.a. Forced swim test

This is a test for evaluating depression-like symptoms. As described previously (Porsolt et al., 2001) mice were placed in transparent glass cylinders (diameter, 10 cm; height, 25 cm) filled up to 10 cm with water (23–25 °C) for 6 min test and scored for duration of immobility. A mouse was considered as immobile when floating motionless or making only those movements necessary to keep its head above the water. The *db/db* and lean control mice were tested simultaneously in separate cylinders with opaque white plexiglass sheet placed between them so that mice could not see each other. During test sessions, mice were video recorded to score immobility time. After the swim test, mice were dried with a towel and returned to the home cage placed on a thermal blanket heated to 37 °C.

4.5.b. Pre-pulse inhibition (PPI) test

This is a test for evaluating psychosis-like behavior. As shown in our previous report (Mach et al., 2008), mice were tested in automated startle chambers (SM100 Startle Monitor System Version 6.12; Hamilton Kinder, Poway, CA) for pre-pulse inhibition (PPI). There were five types of white noise burst stimulus trials: prepulse (70 dB), pulse (85 dB and 100 dB) and pre-pulse prior to pulse (70 dB+85 dB and 70 dB+100 dB) with background noise (60 dB). Each trial type was presented 10 times. Stimuli were presented in random order to avoid order effects and habituation. The inter-trial intervals were varied from 9 to 16 s. Mice were in holders restricting

rearing behavior and placed on pressure-sensor plates transforming movements of the body (jerks) into an analog signal through an interface.

4.5.c. Open field test

An automated open field system with microprocessor and infrared photo-beams (Hamilton Kinder, Motor Monitor Version 3.11; Poway, CA) was used to evaluate locomotor and thigmotactic behavior of mice [44]. The open field consisted of 16×16 in (40.6×40.6 cm) plexiglass square. For analysis, the chamber was divided into central (8×8 in) and peripheral (4 in wide) zones. Each mouse was placed in the center of the open field arena and allowed to explore it for 10 min. During the 10 min test session, the variables of locomotor activity, basic movements (quantified by IR beam interruptions due to larger mouse body movements in the open field), fine movements (defined by IR beam interruptions due to fine movements such as head-twitching, grooming etc) and percent time spent in periphery and central zones were recorded using Motor Monitor software. Open field arena was cleaned with 70% ethanol solution and let dry after testing each mouse.

4.5.d. Elevated plus maze (EPM) test

This is a behavioral paradigm that takes advantage of the conflict behavior of rodents between exploration of a novel area and aversion to open and elevated spaces (Hata et al., 2001; Pellow et al., 1985). The maze is made up of opaque black Plexiglas with opposite facing two open (14×2 inches) and two enclosed arms (14×2×6 inches) connected by a central platform (2×2 inches). The whole maze is raised 30 inches above the floor. Mice were tested on the plus maze in a room with low, indirect incandescent red lighting and very low noise levels. On the day of testing, the

mouse was placed at the center of the maze with head facing an open arm and allowed to explore for 5 min. The number of entries, time spent and distance traveled in each arm were recorded with the help of an automated elevated plus maze system (EPM) (Hamilton Kinder, Version 3.11; Poway, CA). The software was configured to register entry when all four paws of the animal were placed on the arm. The maze was wiped clean with 70% ethanol solution and dried after testing each mouse. Increase in time spent and frequency of open arms entries relative to control mice were considered as indicators of anxiolytic behavior.

4.5.e. Y-maze test

The Y-maze measures a mouse's functional working memory status and exploits the innate tendency to explore novel areas (Ma et al., 2007). The apparatus for Y-maze test was made of 3 acrylic plastic arms (arm dimensions: 3.5 cm×20 cm) at 120 degrees to each other. During test, individual mice were placed on the intersection of 3 arms of the maze and were video recorded for 8 min to score the number and sequence of arm entries. An arm entry was registered when all four paws of mouse were within any of 3 arms. Entries into 3 different arms in succession (e.g. ABC or BCA or CBA or CAB etc) were defined as alternations. Percent Y-maze scores were calculated using the formula:

$$= \frac{\text{Total number of alternations}}{(\text{Total number of entries}-2)} \times 100$$

4.5.f. Fear potentiated startle (FPS) test

Mice were tested in an automated startle chambers (Kinder Scientific, CA) and loosely restrained in holders on pressure-sensor plates transforming body movements (jumps) into an analog signal

through an interface. Mice were acclimated to the chamber for 5 min prior to testing. During testing 60-decibel (dB) noise as background noise was constantly on. The FPS test consisted of three phases: (1) pre-test, (2) training and (3) post-test. Pre-test measures the mouse's initial startle response to 2 different trials i.e. (i) startle stimulus (85 dB white noise startle stimulus) and (ii) startle stimulus with tone (70dB, 30 s., 12 kHz pure tone plus 85 dB white noise) with an inter-trial interval of 1 min. Each trial was repeated 8 times and presented in randomized fashion (total = 16 trials). The % FPS responses for pre-test were calculated using formula:

$$= \frac{\text{Startle stimulus alone trials} - \text{tone plus startle stimulus}}{\text{Startle stimulus alone}} \times 100$$

The training phase was consisted of 20 trials of fear conditioning per day for 2 consecutive days [70dB, 30 s., 12 kHz (conditioned stimulus, CS) tone] that overlaps and terminates with a 500 ms 0.4 mA shock (unconditioned stimulus, US). The shock onset began 29.5 s after the onset of the tone with inter-trial interval between 1-3 min. Post-test consisted of the same paradigm as pre-test but was performed the day after training and the %FPS response for post-test was calculated using the formula as given above. If the mouse learned the response the %FPS score was expected to be amplified in the post-test.

4.6. Brain dissection

After 1 week acclimatization, adult *db/db* (11-12 week old) and age-matched lean control mice were euthanized by decapitation. The brains were removed and immediately immersed in ice cold 0.9% saline. The frontal cortex (FC) and brainstem (BS) were hand dissected. A mouse brain matrix (Ted Pella, Inc) was used to slice the remainder of the brain into 1 mm or 2 mm thick blocks rostral to the interaural line for three more brain regions: amygdala, hippocampus and hypothalamus. All the brain parts were wrapped in aluminum foil and stored at -80 °C until

further dissection. The mouse brain stereotaxic coordinates (Paxinos and Franklin, 2008) were used as reference to dissect out specific brain regions using -20 °C ice blocks.

4.7. Neurochemical studies

4.7.a. Preparation of tissue samples

All the brain regions were homogenized in 0.2N perchloric acid (300 µl) and centrifuged at 24 × 4G at 4°C for 20 minutes. The aliquots of the supernatant were stored at -80 °C until further analysis.

4.7.b. Brain monoamine analysis

As described previously (Macedo et al., 2004) with some modifications, monoamines and DA catabolite concentrations were measured in the specific brain regions of lean control, *db/db* and *db/db* + Rosiglitazone mice. Briefly, DA, 5-HT, NE, HVA and DOPAC were measured in FC, amygdala, hippocampus, hypothalamus and BS using reverse phase HPLC method. Detection was done using an LC-4B amperometric detector (Bioanalytical Systems Inc. BASi) optimized to 0.75 V. Samples were eluted through an ESA C18 column (80 X 4.6 mm X 3 µm, Chelmsford, MA). The mobile phase stock composed of: sodium phosphate (0.02 M), sodium citrate (0.05 M), ethylenediaminetetraacetic acid (EDTA, 3.4 M), diethylamine hydrochloride (0.01 M) and 1-octanesulfonic acid (0.001 M) was prepared in deionized water (up to 1 L). Phosphoric acid was used to adjust the pH to 3.1. Before analysis of sample aliquots, acetonitrile (12 ml), dimethylacetamide (5.5 ml) and 1-octanesulfonic acid (120 mg) were added to 250 ml volume of mobile phase stock. The mobile phase was run through the system having an amperometric detector at fixed flow rate (0.6 ml/min).

Reference standard solution was prepared using known concentrations of DA, 5-HT, NE, HVA and DOPAC (Sigma-Aldrich, USA). The peaks obtained from reference standards were compared with the corresponding tissue sample neurotransmitter amplitudes to determine concentrations of monoamines and catabolites (ng/mg of wet tissue). Tissue homogenization did not alter monoamine or catabolite concentrations. The ratios of DA catabolites and neurotransmitter DA: HVA/DA and DOPAC/DA were calculated as an index of DA usage.

4.8. Western blot analysis

Different isolated brain parts as mentioned above were homogenized on ice in phosphate buffered saline (PBS) containing protease inhibitor (Complete lysis M, Roche diagnostics, Germany). Homogenates were centrifuged (10,000 x g for 10 min, 4 °C) to remove cellular debris. Total protein content was determined in supernatant using bovine serum albumin (BSA) as a standard and BioRad reagent (BioRad, CA). 30 µl of brain-region lysate was added to 30 µl sample loading buffer of composition: 8% SDS, 125 mmol/L Tris-HCl, pH-6.8, 20% glycerol, 0.02% bromophenol blue, 100 mmol/L dithiothreitol and boiled for 6 minutes. Approximately 8-12 µg protein was loaded to 8% SDS-PAGE gel for separation employing electrophoresis. Proteins on gel were then transferred (Bio-Rad transfer apparatus, CA) to a 0.2 µm PVDF membrane (Millipore, MA). The membranes were blocked for 1 hour with 10% non-fat milk made in 10 mM Tris buffered saline with Tween 20 (TBS-T) at room temperature (RT). For analysis, anti-tyrosine hydroxylase (AB152, Millipore, USA) was used made in 5% non-fat milk in TBS-T and incubated for 3-days at 4 °C to probe the membranes (dilution 1:1000). The membranes were washed with TBS-T buffer 3 times for 5 minutes at RT. Later, membranes were incubated with horse radish peroxidase (HRP) conjugated donkey anti-rabbit secondary antibody (Jackson ImmunoResearch, PA) made in TBS-T buffer (1:40,000 dilution) for 1 hour at RT. Blots

were detected using SuperSignal chemiluminescent substrate (Pierce, IL) and visualized in Fujifilm image analyzer (LAS-3000 Image Quant, CA). TH has a molecular weight of 62 kDa. The relative amounts of TH were determined by normalizing to β -actin (molecular weight 43 kDa).

5. Data analysis

The results are presented as group means \pm S.E.M. and analyzed using Graphpad Prism 5 software. Data were analyzed by 2-way ANOVA test. The two variables for 2-way ANOVA test were 'strain' (*db/db* versus lean control) and 'age' of mice (juvenile versus adult). Bonferroni test was used for *post-hoc* comparisons. Unpaired Student t-test was used to analyze differences in body weight, food intake, water intake, % fat mass, % lean mass, % body water, blood glucose and plasma insulin concentrations. Neurochemistry and western-blot data was analyzed by 1-way ANOVA followed by *post-hoc* Bonferroni test. Value of $p \leq 0.05$ was considered significant.

6. Results

6.1. Metabolic parameters

As expected and shown in Table 1, the blood glucose concentrations of *db/db* mice were significantly higher compared to age-matched lean controls ($p < 0.05$, unpaired t-test). There was an age-dependent increase in blood glucose concentration of *db/db* mice (juvenile: 230.38 ± 13.25 mg/dL versus adult: 583.20 ± 35.86 mg/dL). However, blood glucose concentrations of lean control mice remained fairly constant ($p < 0.05$). Food intake, water intake, plasma insulin and % fat mass of *db/db* mice were significantly increased compared to age-matched lean controls ($p < 0.05$, unpaired t-test). However, percent lean mass and percent body water of *db/db* mice were significantly low compared to age-matched lean controls ($p < 0.05$, unpaired t-test).

6.2. Behavioral tests

6.2.a Depression-like behavior of *db/db* mice

As shown in Fig. 1, juvenile as well as adult *db/db* mice were immobile for significantly more time than age-matched lean controls in the 6 min forced swim test [factor ‘strain’ $F(1, 32) = 83.22$, $p < 0.001$]. There was no age-dependent significant change in duration of immobility in lean control as well as *db/db* mice ($p > 0.05$).

6.2.b. Psychosis-like behavior of *db/db* mice

Normal PPI was observed in juvenile *db/db* mice at both intensities of startle stimuli (Fig. 2; $p > 0.05$). However, percent PPI in adult *db/db* mice was significantly lowered with respect to age-matched lean controls with (70 dB + 85 dB) [factor ‘strain’ $F(1, 36) = 9.705$, $p < 0.001$] and (70 dB + 100 dB) stimuli [factor ‘strain’ $F(1, 36) = 8.575$, $p < 0.05$]. Further, there was an age-

dependent decrease in PPI response of *db/db* mice with (70 dB + 85 dB) startle stimuli [factor 'age' $F(1, 36) = 3.474$, $p < 0.05$] but not with (70 dB + 100 dB) stimuli ($p > 0.05$). Three out of 10 juvenile *db/db* mice also showed disruption of PPI for 70 dB + 100 dB (pre-pulse + pulse) startle stimuli, however, no change in PPI response of remaining 7 juvenile *db/db* mice of the group nullified this difference when compared with age-matched lean control group.

6.2.c. Anxiolytic behavior of *db/db* mice

In the elevated plus maze test, there was a significant increase in percent time spent in open arms (Fig. 3a) [factor 'strain' $F(1, 31) = 7.785$, $p < 0.05$, factor 'age' $F(1, 31) = 16.73$, $p < 0.05$] and percent entries into open arms (Fig. 3b) [factor 'strain' $F(1, 31) = 4.337$, $p < 0.05$, factor 'age' $F(1, 31) = 6.066$, $p < 0.05$] in juvenile and adult *db/db* mice as compared to age-matched lean controls. Moreover, *db/db* mice traveled about equivalent distances on open arms compared to age-matched lean controls (Fig. 3c) ($p > 0.05$).

6.2.d. Hypo-locomotive and thigmotaxis behavior of *db/db* mice

Both juvenile and adult *db/db* mice had significantly fewer activity counts for (a) basic movements and (b) fine movements compared to their age-matched lean controls in the open field test (Fig. 4a and b). Both strains also underwent a decrease in these measures over time. Two-way ANOVA showed a significant effect on basic movements [factor 'strain' $F(1, 35) = 74.17$, $p < 0.001$; factor 'age' $F(1, 35) = 52.94$, $p < 0.001$] and fine movements [factor 'strain' $F(1, 35) = 17.94$, $p < 0.05$; factor 'age' $F(1, 35) = 4.497$, $p < 0.01$] of *db/db* mice compared to age-matched lean controls. During the 10 min test session, *db/db* and age-matched lean control mice spent significantly more time in the peripheral than the central zone indicative of

thigmotaxis ($p < 0.05$). However, there was no difference in percent time spent in periphery between *db/db* mice and age-matched lean controls ($p > 0.05$) (Fig. 4c).

6.2.e. Working memory test in *db/db* mice

Percent Y-maze scores of juvenile as well as adult *db/db* mice were not statistically different from age-matched control littermates ($p > 0.05$; Fig. 5). There was a significant decrease in total number of Y-maze arms entries [factor ‘strain’ $F(1, 33) = 12.91$, $p < 0.01$] and alternation score due to decreased activity [factor ‘strain’ $F(1, 33) = 7.469$, $p < 0.05$] of adult *db/db* mice compared to age-matched control mice that led to no change in their percent Y-maze scores ($p > 0.05$).

6.3. Neurochemistry study

6.3.a. Elevated HVA/DA ratio in the frontal cortex of *db/db* mice

The neurotransmitter DA levels in the frontal cortex of *db/db* mice did not change compared to lean control mice (Fig. 6). However, there was a significant increase in the HVA levels in the frontal cortex of *db/db* mice compared to lean control mice (Fig. 7). These changes resulted into a significant increase in the frontal cortex HVA/DA ratio in *db/db* mice compared to lean controls (Fig. 9). Similar results were reported previously using type-2 diabetic female *ob/ob* mice [71].

6.3.b. Decreased HVA/DA and DOPAC/DA ratios in the 29mygdale of *db/db* mice

In contrast to the frontal cortex, the HVA/DA ratios in amygdala were significantly decreased (Fig. 9, $p < 0.05$), a response consistent with decreased use. The concentrations of both DA and

HVA were higher in *db/db* mice compared to lean controls (Fig. 6 and Fig. 7). A dramatic three-fold rise in DA concentration (ng/mg of wet tissue) in amygdala of *db/db* mice was observed [Fig. 6, *db/db* (2.65 ± 0.60) versus lean control (0.86 ± 0.40)]. This was due to a significant decrease in DOPAC/DA ratio in amygdala of *db/db* mice compared to lean control (Fig. 10). However, no changes were seen in the HVA/DA ratios in the hippocampus, hypothalamus and brainstem regions of *db/db* mice compared to lean controls. Significant increases in DA and DOPAC concentrations were seen in brainstem (Fig. 6 and Fig. 8, respectively). However, the DOPAC/DA ratio remains unaffected. Measurement of DOPAC/DA in brain regions other than the amygdala did not show any significant changes (Fig. 10).

6.3.c. Elevated NE and 5-HT levels in selective brain regions of *db/db* mice

There was a significant rise in NE concentrations in the frontal cortex, hypothalamus and brainstem of *db/db* mice compared to lean controls ($p < 0.05$, Fig. 11). However, NE concentrations in the amygdala and hippocampus did not differ from lean controls ($p > 0.05$). The 5-HT concentrations were increased in frontal cortex, amygdala and brainstem of *db/db* mice compared to lean controls ($p < 0.05$, Fig. 12). In contrast, hippocampal and hypothalamic 5-HT concentrations of *db/db* mice remained unchanged compared to lean controls ($p > 0.05$).

6.4. Rosiglitazone treatment

6.4.a. Rosiglitazone treatment controlled diabetic complications in *db/db* mice

As shown in Fig. 13, rosiglitazone significantly lowered hyperglycemia of *db/db* mice over the 5-week duration of treatment ($p < 0.0001$). This was also evident from plasma glucose concentrations of rosiglitazone treated *db/db* mice. Chronic treatment with rosiglitazone has no

significant on the blood glucose concentration of rosiglitazone treated *db/db* mice was not statistically different from age-matched lean control mice ($p < 0.05$; Fig. 13).

6.4.b. Rosiglitazone treatment improved glucose utilization in the *db/db* mice

In the intraperitoneal glucose tolerance test (GTT), 16 h fasted *db/db* mice showed significantly higher blood glucose values compared to age-matched lean controls ($p < 0.0001$; Fig. 14). In *db/db* mice blood glucose concentrations remain significantly elevated compared to lean control mice at all time-points during 120 min blood sampling ($p < 0.0001$; Fig. 14). This led to significantly higher area under the curve (AUC) in *db/db* mice compared to AUC for the lean control group ($p < 0.0001$; Fig. 14). In contrast, rosiglitazone treatment significantly improved glucose handling by *db/db* mice ($p < 0.0001$; Fig. 14). Blood glucose concentrations of rosiglitazone treated *db/db* mice post-16 h fasting (0 Min) were not significantly different from fasted lean control mice ($p > 0.05$; Fig. 14). Moreover, the AUC post intraperitoneal glucose loading for rosiglitazone treated *db/db* mice was significantly decreased compared to *db/db* mice having no treatment ($p < 0.0001$; Fig. 14).

6.4.c. Rosiglitazone reversed depression-like behavior of *db/db* mice

As shown in Fig. 15, *db/db* mice were immobile for significantly longer in the FST compared to age-matched lean controls suggesting the presence of depression-like behavior ($p < 0.05$). These results were in agreement with our previous findings (Fig. 1). Rosiglitazone treatment significantly decreased the duration of immobility of *db/db* mice in FST compared to *db/db* mice having no treatment ($p < 0.05$; Fig. 15). Thus, five weeks of rosiglitazone treatment to *db/db* mice reversed their depression-like behavior. Rosiglitazone treatment of lean control mice also

significantly reduced the duration of immobility in FST in lean mice. However, this effect was independent of the glucose lowering potential of Rosiglitazone (Fig. 13).

6.4.d. Rosiglitazone does not reverse hypolocomotive behavior of *db/db* mice

As expected, *db/db* mice had significantly less activity counts for (a) basic movements and (b) fine movements compared to their age-matched lean controls in the open field test ($p < 0.0001$; Fig. 16). Rosiglitazone treatment did not reverse the hypolocomotive behavior of *db/db* mice. Both (a) basic movements and (b) fine movements of rosiglitazone treated *db/db* mice were not different compared to their age-matched *db/db* mice having no treatment ($p > 0.05$).

6.4.e. Rosiglitazone does not reverse psychosis-like behavior of *db/db* mice

Pre-pulse inhibition (PPI) behavior of 10-11 week old *db/db* mice was significantly disrupted compared to age-matched lean control mice ($p < 0.05$; Fig. 17) suggesting the presence of psychosis-like behavior. However, 5-week treatment with rosiglitazone failed to reverse psychosis-like behavior of *db/db* mice ($p > 0.05$; Fig. 17). Moreover, rosiglitazone significantly disrupted PPI behavior of lean control mice ($p < 0.05$). These results suggest that rosiglitazone treatment induced psychosis-like symptoms in lean control mice without affecting its blood glucose concentration per se.

6.4.f. Rosiglitazone does not alter *db/db* mouse behavior in the fear potentiated startle test

During the post-test, lean control mice had a potentiated fear response ($p < 0.05$; Fig. 18). In contrast, *db/db* mice did not have any such alterations in their behavior in FPS test ($p > 0.05$; Fig. 18). Further, the FPS behavior of rosiglitazone treated *db/db* mice was not different from *db/db*

mice that were on standard chow ($p > 0.05$). However, rosiglitazone treatment of lean control mice resulted in a potentiated fear response ($p < 0.05$; Fig. 18).

6.5. Effect of rosiglitazone on abnormal DA metabolism pattern in *db/db* mouse brain

6.5.a. Rosiglitazone normalized abnormalities in DA metabolism in the frontal cortex of *db/db* mice

There was no change in the DA levels in the frontal cortex of *db/db* mice compared to lean control mice (Fig. 19). However, there was a significant increase in the frontal cortex HVA ratio in *db/db* mice compared to lean controls ($p < 0.05$; Fig. 20). This led to significant increase in the HVA/DA ratio in the frontal cortex of *db/db* mice compared to lean controls (Fig. 22). Similar results were reported previously using leptin-deficient *ob/ob* mice (Harris et al., 1998).

Interestingly, the HVA/DA ratio in the frontal cortex of rosiglitazone-treated *db/db* mice was not different compared to age-matched lean controls (Fig. 22). The changes observed were due to restoration of frontal cortex HVA levels to normal (Fig. 19). Thus, rosiglitazone treatment significantly restored abnormal frontal cortex extraneuronal DA metabolism pattern to normal. However, rosiglitazone treatment failed to normalize intraneuronal DA metabolism pattern (i.e. DOPAC/DA ratio) in the frontal cortex of *db/db* mice.

6.5.b. Rosiglitazone normalized abnormalities in DA metabolism in the 33mygdale of *db/db* mice

In contrast to the frontal cortex, the HVA/DA ratios in amygdala of *db/db* mice were significantly decreased (Fig. 22, $p < 0.05$), a response consistent with decreased DA use. The concentrations of both DA and HVA were higher in *db/db* mice compared to lean controls (Fig.

19 and Fig. 20, respectively). In concordance of these changes, the DOPAC/DA ratios in the amygdala of *db/db* mice were also significantly decreased compared to lean control mice (Fig. 23, $p < 0.05$). In the amygdala, DA and DOPAC levels were significantly increased. However, the DA increase was proportionally higher (~ 3 fold) compared to DOPAC (Fig. 19 and Fig. 21, respectively) that led to a significant decrease in the DOPAC/DA ratio (Fig. 23). Rosiglitazone treatment to normalize hyperglycemia and improve insulin sensitivity reversed these changes. Rosiglitazone treatment significantly restored DA, HVA and DOPAC levels in the amygdala to normal (Fig. 19, 20 and 21 respectively).

There were no changes in the HVA/DA ratios in the hippocampus, hypothalamus and brainstem regions of *db/db* mice compared to lean controls. Significant increases in DA and DOPAC concentrations were seen in the brainstem (Fig. 19 and Fig. 21 respectively) but the DOPAC/DA ratio remained unaffected. Measurement of DOPAC/DA in brain regions other than the amygdala and brainstem did not reveal any significant changes (Fig. 23). Rosiglitazone administration normalized hyperglycemia and significantly increased DA and DOPAC levels in the hypothalamus and brainstem of *db/db* mice (Fig. 19 and Fig. 21 respectively). However, these changes were proportionally similar. Therefore, the DOPAC/DA ratios remain unaffected (Fig. 23). The brainstem also had a significant elevation in HVA levels (Fig. 20) that accounted for a decline in the HVA/DA ratio (Fig. 22). In the hippocampus, rosiglitazone treatment significantly decreased DOPAC/DA ratio (Fig. 23) due to a reduction in DOPAC levels (Fig. 21).

6.5.c. Tyrosine hydroxylase expression pattern in *db/db* mice brain was similar to non-diabetic lean control mice

To examine if hyperglycemia and insulin resistance affects DA biosynthesis pattern, frontal cortex, amygdala and hypothalamus were analyzed for TH expression pattern (Fig. 24, Fig. 25, and Fig. 26 respectively). Compared to age-matched lean controls, TH expression pattern in these brain regions of *db/db* mice was not different ($p > 0.05$). Further, TH expression pattern in the hypothalamus of *db/db* mice were not significantly different compared to age-matched lean controls ($p > 0.05$; Fig. 26). These results were consistent with no differences in the DA metabolism in the hypothalamus of *db/db* mice compared to age-matched lean controls.

6.5.d. Rosiglitazone normalized abnormalities in adrenal catecholamines in *db/db* mice

To understand the impact of hyperglycemia and insulin resistance on adrenal catecholamine levels in *db/db* mice and effect of improving insulin sensitivity and normalizing hyperglycemia on them adrenal NE, DA and epinephrine (E) concentrations were determined. There was a significant increase in the adrenal NE, E and DA concentrations in *db/db* mice compared to age-matched lean controls (Fig. 27). In contrast, normalization of hyperglycemia and improving insulin sensitivity using 5-week rosiglitazone treatment helped to restore *db/db* mice adrenal catecholamine levels to normal (Fig. 27).

7. Discussion

Type-2 diabetes significantly affects socio-economic status, quality of life and physical and mental health of diabetic patients. Neuropsychiatric disorders such as depression and schizophrenia are frequent co-morbid conditions in the diabetic population. The neuropsychological consequences of diabetes have been studied with reasonable detail in the clinical setting. However, there is still a scarcity of preclinical research supporting correlations between type-2 diabetes and such consequences. The current study attempted to provide experimental evidence for an interrelationship between type-2 diabetes and neuropsychological deficits using *db/db* mice. The principal findings of this study are:

- (1) Behavioral depression, age-related advancement of psychosis-like symptoms and anxiolytic behavior in *db/db* mice.
- (2) Abnormal DA metabolism patterns in the frontal cortex and amygdala of *db/db* mice that correlated with their behavioral deficits.
- (3) Rosiglitazone-induced reversal of depression- but not psychosis-like behavior and impaired emotional learning in *db/db* mice.
- (4) Restoration of the majority of changes observed in DA metabolism in the brains of *db/db* mice to normal post-rosiglitazone treatment.
- (5) Significant elevation in the adrenal catecholamine levels in *db/db* mice compared to age-matched lean controls. Rosiglitazone treatment helped to restore them to normal.

7.1. Neurobehavioral deficits in *db/db* mice

Depression is the major co-morbid psychological disorder with diabetes (Anderson et al., 2001; Lin et al., 2004). We evaluated *juvenile* and *adult db/db* mice for behavioral depression using the forced swim test (Fig. 1). Both age-groups of *db/db* mice showed significant increases in the

duration of immobility compared to age-matched lean control mice suggesting the occurrence of depression-like symptoms in *db/db* mice. While the obesity of the *adults* may have impaired swimming ability, the swimming deficits in non-obese *juveniles* suggest that central nervous system mechanisms related to depression are the cause. Additionally, poor thermoregulation in *db/db* mice can also confound immobility duration in FST. However, Trayhurn reported similar diurnal rhythms in body temperature of *db/db* mice compared with control mice at 23 °C (Trayhurn, 1979). We used a similar temperature range (23–25 °C) for FST. In contrast to rats that generally require two-trial FST, one-trial is adequate to produce consistent immobility scores using mice in FST (Borsini and Meli, 1988; Cryan and Lucki, 2000; Lucki et al., 2001).

The probability of depression in diabetic patients is approximately double that of those without diabetes (Anderson et al., 2001). Behavioral despair in diabetic patients can lead to apathy towards self-care regimens such as regular physical exercise, dietary habits, cessation of smoking and towards medication for diabetes (Lin et al., 2004). Diabetic patients with comorbid depression are reported to have double the probability of cardiac risk factors like smoking, obesity, sedentary lifestyle etc (Katon et al., 2004). Recently Hirano and colleagues reported a decrease in circulating plasma leptin concentrations and depression-like symptoms in streptozotocin-induced diabetic mice (Hirano et al., 2007). Interestingly, treatment of these diabetic mice with leptin reversed the depression-like behavior in the tail suspension test (TST), a model for depression. In view of the mutation in leptin receptors (LRb) in *db/db* mice and an antidepressant-like effect of leptin in a mouse model for diabetes, behavioral depression observed in *db/db* mice may be the outcome of impaired leptin signaling. Leptin treatment restores the preference for sucrose consumption in rodents subjected to chronic stress and reduced preference for sucrose is analogous to anhedonia, a hallmark feature of depression

(Katz, 1982; Lu et al., 2006). Further, systemic leptin treatment is reported to lower immobility in FST and TST in rodents, an indicator of antidepressant-like effect of leptin that was not dependent upon stimulation of motor behavior. Thus, leptin can serve as a potential neurobiological substrate for the treatment of depression (Lu et al., 2006). Several research groups reported expression of leptin receptors in serotonergic raphe nuclei (Finn et al., 2001) and dopaminergic ventral tegmental area and substantia nigra (Figlewicz et al., 2003). Collin and co-workers reported a fall in serotonin transporter mRNA expression in raphe nuclei of functional leptin deficient *ob/ob* mice (Collin et al., 2000). Leptin increases production of serotonin and its biotransformation product, 5-HIAA in the forebrain (Calapai et al., 1999). Thus, it is plausible that leptin may interact with monoaminergic neurons involved in the pathophysiology of depression and related disorders by modulating their firing pattern and downstream signaling mechanisms in a manner that may lead to depression in humans and to the behavioral deficits in *db/db* mice.

We used PPI of startle to evaluate age-dependent progression of psychosis-like symptoms in *db/db* mice (Fig. 2). While *juvenile db/db* mice did not differ from age-matched lean controls in terms of percent PPI scores when subjected to a series of randomly presented pre-pulse plus pulse (70 dB + 85 dB and 70 dB + 100 dB) startle stimuli trials, *adult db/db* mice experienced significant disruption of PPI behavior with respect to age-matched lean controls. Such disruption of PPI behavior in *adult* but not in *juvenile db/db* mice signifies age-dependent progression of psychosis-like behavior in this murine model for type-2 diabetes. Three out of 10 *juvenile db/db* mice also showed psychosis-like behavior, which may indicate that this is the age at which abnormalities begin to develop. PPI behavior is a natural hardwired trait of the normal, non-

psychotic subjects. Disruption of PPI is the hallmark symptom to confirm psychosis-like symptoms and it is rare to ever have a disruption in a normal animal.

Comorbid type-2 diabetes is reported to impair the physical as well as mental health status of adult schizophrenic patients compared to non-diabetic schizophrenics (Dickinson et al., 2008). The emergence of new-onset diabetes was reported in schizophrenic patients following initiation of atypical antipsychotic medications (Lambert et al., 2006; Ramaswamy et al., 2006; van Winkel et al., 2008). Hyperglycemia and insulin resistance was also reported in mice that were on chronic antipsychotic treatment (Dwyer and Donohoe, 2003). Current clinically proven medications for psychosis may not prove a good strategy to treat co-occurring psychotic symptoms in the diabetic population because they may further complicate existing diabetic phenotypes. Recently van Nimwegen and co-workers showed that antipsychotic naive schizophrenics also have hepatic insulin resistance compared to non-schizophrenic controls. Such increased propensity towards insulin resistance in schizophrenics may be the outcome of metabolic abnormalities (van Nimwegen et al., 2008). These findings hint toward a bidirectional link between diabetes and schizophrenia.

We observed *db/db* mice to be less anxious than age-matched lean controls in the elevated plus maze test (Fig. 3). *Juvenile* as well as *adult db/db* mice spent significantly more time on the open arms compared to age matched lean controls. *db/db* mice also showed a significant increase in percent open arms entries compared to age-matched lean controls. Both age-group *db/db* mice traveled equal distances on open arms compared to age-matched lean controls suggesting that the observed increase in time spent into open arms by *db/db* mice is not affected by their hypo-locomotive behavior. Though *db/db* mice have impaired leptin signaling, previous literature

about leptin's role in anxiety behavior is ambiguous. Asakawa and co-workers reported that leptin treatment can ameliorate anxiety-like behavior of *ob/ob* mice (Asakawa et al., 2003). On the other hand, Buyse and colleagues reported decreased open arm exploration in the elevated plus maze test after intraperitoneal leptin treatment in diet-restricted rats (Buyse et al., 2001). In contrast, Suomalainen and Mannisto reported that higher doses of leptin (10 and 20 mg/kg) failed to affect anxiety behavior in *ad libitum* fed mice (Suomalainen and Mannisto, 1998). Also, in spontaneously diabetic INS2^{Akita} mice, Asakawa and colleagues reported anxiety-like behavior using the elevated plus maze test (Asakawa et al., 2007). Labad and colleagues recently have shown that depression but not anxiety is associated with type-2 diabetes (Labad et al., 2010). Results of anxiety and depression screening of *db/db* mice are consistent with this clinical report showing the presence of depression but not anxiety. In contrast to this, high anxiety scores (Collins et al., 2009) and an increased prevalence of generalized anxiety disorder was reported in type-2 diabetic patients relative to non-diabetic adults elsewhere (Fisher et al., 2008). Clinical studies evaluating anxiety levels in type-2 diabetic patients led to conflicting outcomes. Exploring the biological mechanisms that govern anxiolytic behavior of *db/db* mice may help to understand why there are differences in some but not all neuropsychological deficits with type-2 diabetes.

In the open field test, *db/db* mice undergo a decrease in basic movements (quantified by IR beam interruptions due to more body movements in the open field) and fine movements (IR beam interruptions due to fine movements such as head-twitching, grooming etc) compared to age-matched lean controls (Fig. 4). These results are in agreement of our previous research report (Senador et al., 2009) and others (Laposky et al., 2008; Stranahan et al., 2009). Laposky and colleagues used 3–4 months (or 12–16 weeks) *adult db/db* mice and studied total activity counts,

while the present study investigated locomotor behavior of much younger (5–6 weeks) *db/db* mice. Similar changes in locomotor behavior of *juvenile db/db* mice were reported by Hesse and co-workers (Hesse et al., 2009). We evaluated *db/db* mice for basic movements due to whole body movements as well as fine movements resulting from head-twitching, grooming etc. Hypo-locomotion is the hallmark feature of several psychiatric disorders like Parkinson's disease, Huntington chorea, antipsychotic-induced pseudoparkinsonism etc. While some have argued that decreased locomotor activity in the open field test may account for increased duration of immobility in FST, others have observed no correlation between locomotor activity and performance of mice in FST (Collin et al., 2000; Crawley et al., 1997). Increased immobility in FST as well as decreased locomotor activity was reported in *ob/ob* mice (Collin et al., 2000). Further, clinical studies indicated comorbidity of depression with hypo-locomotive disorders such as Parkinsonism (Papapetropoulos et al., 2006). If a decrease in locomotor activity is causing increased immobility time in the forced swim test, it should also reduce the distance traveled on open arms in the elevated plus maze. However, there was no reduction in the *db/db* mice compared to age-matched lean controls in distance traveled. We also believe that the open field test is not a behavioral model to test depression-like symptoms unless the animals are olfactory bulbectomized. It is most appropriately used to reflect deficits in motor behavior and to test partial anxiety than depression of mice with intact olfactory bulbs. Thus, depressive-symptoms can also be observed in hypo-locomotive individuals. The use of alternative models such as learned helplessness, sucrose preference test or TST that mimics depression-like symptoms may further strengthen the evidence for the presence of depressive symptoms in this mouse strain. Unfortunately the sucrose consumption test requires an extensive amount of time before screening for depression-like behavior (Strekalova et al., 2004) which may preclude

evaluation of age-related changes in behavior. Previous reports suggest that TST is not the ideal test for mice with C57 genetic background (like *db/db* mice) that have a tendency to climb on the tail (Mayorga and Lucki, 2001). Further, TST, like FST, can also depend on motor activity (Cryan and Lucki, 2000). Like lean controls, *db/db* mice also exhibited thigmotaxis behavior—a tendency of animals to stay in proximity of support while traveling. This is indicative of a normal response to the low stress environment of an open arena. Stranahan and colleagues also showed similar tendency of *db/db* mice to spend less time in the center arena. However, they also have reported significant difference in percent time in center arena between *db/db* mice and control mice (Stranahan et al., 2009).

Cognitive deficits are commonly seen in obese and diabetic rodents (Li et al., 2002) and are also prevalent in type-2 diabetic patients (Gispén and Biessels, 2000). Diabetic obese rodents have impaired spatial memory task performance in the Morris water maze test (Li et al., 2002). We tested *db/db* mice for their working memory rather than spatial memory performance. Both age-group *db/db* mice did not show signs of impairment of working memory in Y-maze test compared to age-matched lean control mice (Fig. 5). While the frontal cortex is the anatomical site for working memory, the hippocampus's role as the regulator for spatial memory functions is well documented (Bohlen et al., 2006; Courtney et al., 1998). Recently, Stranahan and colleagues reported diabetes-induced detrimental effects on hippocampal neurons, impaired recovery from hippocampal neuronal defects post-calorie restriction and increased energy expenditure in insulin-resistant and leptin-receptor mutated *db/db* mice (Stranahan et al., 2009). Further, leptin can alter neuronal dendrite morphology (O'Malley et al., 2007). Elevations in corticosterone levels (Stranahan et al., 2008) as well as reduced hippocampal brain-derived neurotrophic factor (BDNF) levels (Stranahan et al., 2009) are suggested as crucial mechanisms

for diabetes-related cognitive deficits. Ohta and colleagues using conditioned taste aversion (CTA) learning test showed that impaired downstream signaling due to the mutation in leptin receptors in *db/db* mice had no effect on acquisition of CTA learning but promoted faster extinction (Ohta et al., 2003). Yamamoto suggested the parabrachial nucleus, amygdala, insular cortex, supramammillary nucleus, nucleus accumbens, and ventral pallidum as possible anatomical sites for CTA learning in rats (Yamamoto, 2007). Li and colleagues reported compromised spatial memory performance in the Morris water maze test (Li et al., 2002). *db/db* mice may have intact working memory as evident from Y-maze test results but poor spatial memory performance and faster extinction of CTA points to anatomical specificity in memory deficits.

Apart from leptin and insulin resistance (de la Monte et al., 2009), hormones like ghrelin (Sun et al., 2007) and neurotransmitters e.g. norepinephrine (Garris, 1988; Garris, 1995) could be other mechanisms that may govern type-2 diabetes-linked CNS dysfunctions. Evidence suggests that leptin can restore normal glucose levels (Pelleymounter et al., 1995) and improve insulin-sensitivity of target organs (Levin et al., 1996; Lin et al., 2002). In contrast, long-term hyperglycemia lowers leptin levels (Moriya et al., 1999). Thus insulin and leptin may have similar mechanisms and maintain close temporal synchronism. Although mutations in leptin receptors are not observed clinically, *db/db* mice could be an important experimental tool to investigate the possible involvement of leptin in diabetes related insulin resistance and comorbid complications. Recently, Schwartz and Bahn reviewed possible application of altered proteins and metabolites identified in schizophrenic brain cerebrospinal fluid (Schwarz and Bahn, 2008). Investigations for such schizophrenia-specific biomarkers in type-2 diabetic brains may unravel mechanisms that led to age-dependent advancement of psychosis-like behavior in *db/db* mice. In

view of resistance to insulin (severe hyperinsulinemia) and leptin (mutation in leptin receptor LRb isoform), pharmacological manipulation of *db/db* mice with exogenous insulin or leptin to correct observed behavioral depression and psychosis-like symptoms do not seem to have a convincing rationale.

In conclusion, this is the first study to report behavioral depression, psychosis-like symptoms and anxiolytic behavior in *db/db* mice strain. Also, *db/db* mice were hypo-locomotive; exhibited normal thigmotaxis and no impairment of working memory. Thus, *db/db* mice showed the presence of select group of neuropsychological deficits. The *db/db* mice could be used as a model to study type-2 diabetes-induced depression and psychosis. Investigations to find biochemical and neurobiological markers governing these deficits may help to improve our understanding about interrelationships between diabetes and comorbid CNS disorders.

7.2. Impact of type-2 diabetes on the brain dopamine metabolism in *db/db* mice

Although clinical studies suggest an increased prevalence of neuropsychiatric disorders in type-2 diabetic patients, the primary mechanisms for such co-morbidities are poorly understood. There is a need to study the neurochemical basis of such co-morbidities employing type-2 diabetic animal models to increase our understanding of their interrelationships. Classic theories of major neurobehavioral disorders suggest changes in the usage of dopamine (DA) in specific brain regions (Howes and Kapur, 2009; Wise, 2008). DA is one of the key neurotransmitters that is involved in the pathophysiology of schizophrenia (Howes and Kapur, 2009) and depression (Dunlop and Nemeroff, 2007). Based on this, the *db/db* mouse brain regions that were selected for analysis of changes in DA turnover receive DA neuronal projections from distinct neuroanatomical dopaminergic pathways with different physiological and pathophysiological

significance: e.g. mesolimbic-mesocortical, nigrostriatal, periventricular, incerto-hypothalamic and descending DAergic systems (Farooqui et al., 1994). The principal finding of the neurochemical experiments is that insulin resistance and hyperglycemia in *db/db* mouse differentially influences DA turnover in different brain regions. To our knowledge, this is the first evidence for elevated HVA/DA ratios in *db/db* mouse brain frontal cortex and this is consistent with our recent findings of disrupted pre-pulse inhibition behavior in this strain analogous to psychosis-like symptoms (Sharma et al., 2010a; Sharma et al., 2010b). The observed elevation in HVA/DA ratio in *db/db* mice frontal cortex was the result of an increase in the HVA concentration, which is only formed by extra-neuronal biotransformation. The frontal cortex receives neuronal projections from the mesolimbic-mesocortical DAergic system and DA neurotransmission in this brain region plays a distinctive role in the modulation of the startle reflex as measured by pre-pulse inhibition of the reflex (Lazar et al., 2008; Mehler-Wex et al., 2006; Nakasato et al., 2008). The increased HVA concentration in the frontal cortex of *db/db* mice parallels that of post-mortem increases in frontal cortex HVA levels in schizophrenic patients compared to normal brains. (Bacopoulos et al., 1979; Davis et al., 1991). No changes in TH expression pattern were seen in the frontal cortex of *db/db* mice compared to age-matched lean controls. Thus, abnormal DA metabolism pattern in this brain region of *db/db* mice may be related to changes in the enzymatic activity of DA metabolizing enzymes and/or altered neuronal firing.

The amygdala is an important component of the mesolimbic DAergic system composed of neuronal projections from the ventral tegmental area (VTA), retrorubral field and substantia nigra pars compacta (SNpc) in the mesencephalon to the amygdala, nucleus accumbens and hippocampus. DA neurotransmission in the amygdala plays an important role in conditioned fear

(Fadok et al., 2009) and pre-pulse inhibition (Stevenson and Gratton, 2004). In the present study, we observed significantly increased DA and HVA concentrations in *db/db* mice amygdala compared to lean controls. However, a greater increase in DA than HVA was observed (HVA: 51% increase versus DA: 231% increase). The DOPAC concentrations in the amygdala of *db/db* mice remained unchanged compared to lean controls. Altogether, these changes led to a significant decrease in amygdala HVA/DA and DOPAC/DA ratios. Further, there were no changes in the TH expression pattern in the amygdala of *db/db* mice compared to age-matched lean controls. Thus, an abnormal DA metabolism pattern in the amygdala of *db/db* mice may be related to changes in the enzymatic activity of DA metabolizing enzymes. Nonetheless, such large increases in both the parent transmitter and its extracellular catabolite warrant further investigation for expression of DA metabolizing enzymes and neuronal firing patterns in this brain region.

The hippocampus receives mesolimbic DAergic projections and is involved in cognitive functions and pre-pulse inhibition behavior (Ellenbroek et al., 2002). We did not observe any significant differences in DA, HVA or DOPAC concentrations in the hippocampus. This led to no changes in HVA/DA and DOPAC/DA ratios in the hippocampus of *db/db* mice compared to lean control mice.

The hypothalamus receives DAergic projections from the incerto-hypothalamic DAergic pathway with the important function in regulation of feeding behavior. The BS also receives descending DAergic neuronal projections yet other cell bodies. We did not observe any significant differences in HVA/DA and DOPAC/DA ratios in hypothalamus or BS of *db/db* mice compared to lean controls. Thus, the type-2 diabetes-induced changes in DA function are brain-

region and DAergic-pathway specific, as would be observed in specific changes in either a subset of DA cell bodies or in the neuronal input to these cell bodies.

This is the first experimental evidence of altered DA neurotransmission in distinct brain regions of *db/db* mice. In clinical practice, the interrelationship between type-2 diabetes and the DAergic systems is gaining increased attention. Recently, the FDA approved the DA receptor agonist, bromocriptine, for treatment of type-2 diabetes to improve glycemic control. The current literature also suggests crosstalk between DA receptors and insulin secretion (Contreras et al., 2008a; Garcia-Tornadu et al., 2010b). In addition to DA, leptin (Venkatasubramanian et al., 2010) and insulin (Guest et al., 2011) implicated in development of neuropsychiatric disorders. Research examining the role of such novel candidates in type-2 diabetes related CNS dysfunctions may help to explain mechanisms governing behavioral and neurochemical alterations in *db/db* mice.

7.3. Impact of improved insulin sensitivity and normalized hyperglycemia on neurobehavioral deficits in *db/db* mice

We observed depression- and psychosis-like behaviors in *db/db* mice (Fig. 1 and Fig. 2, respectively). These findings were in agreement with clinical findings of increased prevalence of depression (Anderson et al., 2001) and schizophrenia (Mukherjee et al., 1996; Mukherjee et al., 1989) in type-2 diabetic population. To extend our understanding of the mechanisms underlying type-2 diabetes associated neurobehavioral deficits, we treated *db/db* mice with the insulin sensitizer, rosiglitazone. The principal findings of this study are: insulin resistance and related hyperglycemia are the underlying mechanisms for depression but not psychosis-like behavior of *db/db* mice (Fig. 15 and Fig. 17, respectively).

A meta-analysis of clinical studies concludes that depression in the diabetic population is linked to hyperglycemia (Lustman et al., 2000). Drugs targeting insulin resistance and hyperglycemia such as pioglitazone have beneficial effects on brain functions in humans and in experimental models (Thal et al., 2011). Pioglitazone injection into C57BL6 mice post-cortical injury dose-dependently reduced brain damage and inflammation (Thal et al., 2011). Rosiglitazone decreases the severity of symptoms in insulin-resistant depressed patients (Rasgon et al., 2010). Based on these studies, we subjected juvenile *db/db* mice to 5-weeks of rosiglitazone treatment and studied its impact on the neurobehavioral deficits. Rosiglitazone normalized hyperglycemia to age-matched lean controls (Fig. 13). Moreover, rosiglitazone treated *db/db* mice handled glucose more efficiently than those without treatment (Fig. 14) indicating improved insulin sensitivity.

Rosiglitazone treated *db/db* mice exhibited a significant increase in the duration of mobility in the forced swim test. Thus, rosiglitazone treatment helped to reverse depression-like behavior of *db/db* mice (Fig. 15). However, the motor behavior of rosiglitazone treated *db/db* mice was not different from *db/db* mice that were on standard chow (Fig. 16). In the open field test, basic movement and fine movement of rosiglitazone treated *db/db* mice were not significantly different compared to *db/db* mice fed with standard chow. Thus, the rosiglitazone-induced decrease in immobility time in the FST was independent of changes in locomotor activity. Eissa Ahmed and co-workers reported antidepressant-like effect of rosiglitazone in rats and mice (Eissa Ahmed et al., 2009). However, they used normal non-diabetic strains for their study. We observed a similar antidepressant-like effect of rosiglitazone in lean control mice that was independent of its antihyperglycemic effect. Administration of the PPAR γ receptor agonist pioglitazone decreased the duration of immobility in mouse FST and prior administration of a PPAR γ antagonist attenuated the effect (Sadaghiani et al., 2011). These results suggest that

PPAR γ receptor activation may mediate antidepressant-like effects of rosiglitazone in lean controls independently of its effect on blood glucose. Neuron-specific PPAR γ receptor knockout and high fat diet mice underwent very little improvement in glucose metabolism after rosiglitazone (Lu et al., 2011), suggesting role for central PPAR γ receptors in hepatic insulin sensitivity. Thus, we speculate that diabetes related hyperglycemia and insulin resistance at least partially contribute to the development of depression-like behavior in *db/db* mice.

To study the effect of normalizing hyperglycemia and improving the insulin sensitivity of psychosis-like behavior of *db/db* mice, we used the pre-pulse inhibition (PPI) of startle test. As shown in Fig. 2, *db/db* mice had an age-dependent advancement of psychosis-like behavior. A separate group of adult *db/db* mice also showed disruption of PPI behavior compared to age-matched lean controls (Fig. 17) thus replicating the finding of psychosis-like behavior. In contrast to its antidepressant-like effect in FST, rosiglitazone treatment did not restore PPI behavior in *db/db* mice (Fig. 17). Thus, controlling hyperglycemia and insulin resistance failed to overcome the psychosis-like behavior of *db/db* mice. However, rosiglitazone significantly disrupted PPI behavior of lean control mice (Fig. 17). A growing amount of evidence suggests an important role of leptin in psychiatric disorders (Sentissi et al., 2008; Zupancic and Mahajan, 2011). Clinical studies indicate that antipsychotic drugs increase systemic leptin levels (Sentissi et al., 2008) and this may be the crucial mechanism for their antipsychotic action. Rosiglitazone treatment lowers systemic leptin levels in lean control mice (Holguin et al., 2007) and this may be the mechanism for the induction of psychosis-like behavior in lean controls. Maeda and co-workers used a psychostimulant-induced behavioral sensitization model to study the effect of PPAR γ receptor agonists on psychosis behavior in mice (Maeda et al., 2007). Methamphetamine-induced behavioral sensitization in mice is a neurobiological model for psychostimulant-induced

psychosis behavior. PPAR γ agonists reversed methamphetamine withdrawal symptoms but not the development of behavioral sensitization to methamphetamine in mice (Maeda et al., 2007). Thus, PPAR γ receptors regulate behavioral sensitization to methamphetamine in mice. *db/db* mice are refractory to leptin treatment because of the mutation in the functional leptin receptor isoform LRB (Chen et al., 1996). Perhaps, alternative mechanisms like impaired leptin signaling may be the dominant mechanism governing psychosis-like behavior of *db/db* mice. Evaluation of psychosis-like behavior in the alternative mouse strain like *ob/ob* mice that responds to leptin treatment may help to answer this question. Further investigations for changes in schizophrenia-specific diagnostic markers, as reported elsewhere (Schwarz and Bahn, 2008), in type-2 diabetic brains may help to unravel mechanisms for psychosis-like behavior in *db/db* mice.

Stress may be an important contributing factor to the development of diabetes and comorbid disorders (Castaneda et al., 2011; Trento et al., 2010). There is impaired glucose tolerance in a mouse model for post-traumatic stress disorder (PTSD) (Castaneda et al., 2011). In a recent cross-sectional study, there was a strong association between lifetime PTSD symptoms in low-income patients and > 7% HbA_{1C} levels (Miller et al., 2011). Alternatively, PTSD is a risk factor for the development of diabetes (Weiss et al., 2011). Based on these findings, we evaluated *db/db* mice for PTSD-like behavior using the fear-potentiated startle (FPS) test. The FPS test is a neurobehavioral model involving fear conditioning in which a neutral stimulus is paired with an aversive event in which the animal exhibits an exaggerated startle response in the presence of the conditioned stimulus alone (Davis, 1993; Maren, 2001a; Maren, 2001b). Abnormalities in the amygdala DA neurotransmission can interfere with fear conditioning response. *db/db* mice showed impairment in acquiring FPS compared to age-matched lean controls (Fig. 18). These results were consistent with the neurochemical finding of impaired DA

metabolism in the amygdala of *db/db* mice (Fig. 22 and Fig. 23). However, controlling hyperglycemia by means of rosiglitazone treatment did not alter impaired FPS learning behavior of *db/db* mice. Rosiglitazone treatment did not affect blood glucose concentration of lean control mice (Fig. 13) but it caused impairment in their FPS learning behavior (Fig. 18). Thus, Rosiglitazone treatment itself may be a risk factor in heightening PTSD symptoms.

Thus, early pharmacological intervention to improve insulin sensitivity and normalize hyperglycemia qualifies to overcome type-2 diabetes related selective neurobehavioral deficits such as depression. Further, glutamate (Widerlov et al., 1988), dopamine (Davis et al., 1991; Dunlop and Nemeroff, 2007), serotonin (Butler and Meegan, 2008) and neuropeptides (Yasuhara and Chaki, 2010) are the dominant mechanisms orchestrating neuropsychiatric disorders. Such alternative mechanisms may contribute to type-2 diabetes related neurobehavioral deficits. Recently, the neurotransmitter dopamine was identified as having a role in type-2 diabetes and related complications (Barber et al., 2003; Chen and Yang, 1991; Cincotta et al., 1997; Contreras et al., 2008b; Fetissov et al., 2002; Figlewicz et al., 1996; Gainetdinov, 2007; Garcia-Tornadu et al., 2010a). Thus, deficiencies in dopamine signaling may contribute to the progression of type-2 diabetes.

7.4. Impact of improved insulin sensitivity and normalized hyperglycemia on abnormalities in brain DA metabolism pattern in *db/db* mice

To test the hypothesis that hyperglycemia and insulin resistance are the mechanisms for abnormal DA metabolism in *db/db* mouse brain, mice were subjected to 5 weeks of rosiglitazone treatment. Rosiglitazone treatment reduced frontal cortex HVA levels to those that were not different from age-matched lean controls. This accounted for restoration of the HVA/DA ratio to normal (Fig. 9 and Fig. 22). Further, long-term rosiglitazone treatment significantly restored

DA, HVA and DOPAC levels in the amygdala to those of age-matched lean controls (Fig. 19, 20 and 21, respectively). Thus, we speculate that hyperglycemia and insulin resistance might be the causative mechanisms for abnormal DA metabolism pattern in the frontal cortex and amygdala of *db/db* mice. In the hippocampus, rosiglitazone treatment significantly reduced hippocampal DOPAC levels and therefore DOPAC/DA ratio suggesting rosiglitazone-induced decreased DA usage in this brain region. Further, rosiglitazone treatment significantly increased DA and DOPAC concentrations in the hypothalamus. These changes accounted for decreased HVA/DA and DOPAC/DA ratios in this brain region of *db/db* mice. Thus, the type-2 diabetes-induced changes in DA function are brain-region and dopaminergic pathway specific, as would be observed in specific changes in either a subset of DA cell bodies or in the neuronal input to these cell bodies. In the brainstem, rosiglitazone treatment caused lowering of HVA/DA and DOPAC/DA ratios in *db/db* mice compared to lean controls. This effect was observed due to rosiglitazone-induced increase in DA, HVA and DOPAC levels in the brainstem of *db/db* mice. Thus, rosiglitazone treatment increased brainstem dopaminergic activity.

7.5. Type-2 diabetes related alterations in the NE and 5-HT concentrations in selected brain regions of *db/db* mice.

In addition to DA, the role of NEergic and 5-HTergic systems in neuropsychological deficits is well documented (Blows, 2000a; Blows, 2000b). The central NE system has two major neuronal projections. One of them has origin in the later ventral tegmental NEergic cell bodies with projection to the forebrain and plays a crucial role in aggressive and feeding behaviors (Bhatia et al., 1997; Hoebel et al., 1989). The second NEergic system originates from the locus coeruleus (LC) in BS and innervates the cerebellum, thalamus, hypothalamus and midbrain with diverse

biological functions. NE transporters are the mechanism for termination of synaptic NE action in the brain. One recent study suggested that aberrations in downstream insulin signaling proteins, normally observed in diabetes, significantly decreases expression of NE transporters in the brain (Robertson et al., 2010). Consistent with this, we observed significantly increased NE concentrations in FC, hypothalamus and BS regions of *db/db* mice compared to lean controls in agreement with previous studies (Garris, 1990; Garris, 1995). NEergic hyperactivity in *db/db* mouse brain is consistent with depression-like behavior of this mouse strain (Sharma et al., 2010a; Sharma et al., 2010b). Clinical and preclinical studies also support NEergic hyperactivity in depression (Gold and Chrousos, 2002; Simson and Weiss, 1988a; Simson and Weiss, 1988b). Further, NE in the FC plays a critical role in mesoaccumbens DA release and reward (Ventura et al., 2003). Hypothalamic NE is implicated in regulation of neural circuits involved in eating disorders (Shor-Posner et al., 1985) while the LC nucleus has an important role in depression and anxiety. Increase in hypothalamic NE concentration was considered as a crucial mechanism for diabetes related neuroendocrine abnormalities such as hyperphagic and polydipsic behavior (Barber et al., 2003). Thus, elevated NE levels in the selective brain regions of *db/db* mice corroborated with their neurobehavioral deficits.

5-HT within the FC is suggested to modulate cognition and emotion (Zhong et al., 2008). Selective 5-HT depletion in the FC can produce a behavioral phenotype similar to obsessive-compulsive disorder and schizophrenia (Clarke et al., 2005). We observed an elevated 5-HT concentration in FC even though they exhibited psychosis-like symptoms. Although conflicting roles for 5-HT in FC are reported, we speculate that diabetes-related psychosis-like symptoms may be related to a deficit in 5-HT release causing elevated levels. Alternatively, the levels are independent of the symptoms. Measurement of its metabolite, 5-HIAA, or microdialysis

experiments would be necessary to resolve this question. The amygdala receives large 5-HTergic innervations from the dorsal raphe nucleus of the BS and activation of this nucleus elevates 5-HT release in the amygdala (Chaouloff, 2000). We observed a significant rise in the 5-HT concentration in the amygdala of *db/db* mice compared to lean controls. The BS contains a cluster of 5-HTergic neurons with diverse physiological functions that includes wakefulness, thermoregulation and regulation of blood pressure (Brodie and Shore, 1957). Although 5-HT is distributed throughout in the brain, its highest concentration is in the BS where it acts as a mediator for certain subcortical centers. Thus, elevated 5-HT concentrations in the BS of *db/db* mice may participate in attention deficits, poor thermoregulation and poor blood pressure regulation. No changes in hypothalamic 5-HT concentrations were seen in *db/db* mice compared to lean controls which is in agreement with previous a report using same mouse strain (King and Rohrbach, 1990). It would be interesting to pursue future studies that may address the neuropathological significance of differential alterations in NE and 5-HT function instead of just their concentrations in discrete brain regions of *db/db* but not lean control mice.

7.6. Type-2 diabetes related alterations in the adrenal catecholamines in *db/db* mice

Long-term stress management in type-2 diabetic patients has been shown to improve glycemic control in clinical setting (Surwit et al., 2002). Further, diabetes has long been considered as a stress disorder (Golbidi et al., 2011). To understand the impact of hyperglycemia and insulin resistance on adrenal catecholamine levels in *db/db* mice and the effect of improving insulin sensitivity and normalizing hyperglycemia on them adrenal NE, DA and epinephrine (E) concentrations were determined. There was a significant increase in the adrenal NE, E and DA levels in *db/db* mice compared to age-matched lean controls (Fig. 27). Further, normalization of

hyperglycemia and improving insulin sensitivity using 5-week of rosiglitazone treatment helped to restore *db/db* mice adrenal catecholamine levels to normal (Fig. 27). Hypothalamic DA, NE concentrations and TH expression of *db/db* mice were not statistically different compared to age-matched lean controls (Fig. 6 and Fig. 11). A compelling amount of evidence supports a role of stress-induced sympathetic system over-stimulation in the pathophysiology of type-2 diabetes (Surwit and Feinglos, 1988; Surwit and Schneider, 1993) consistent with the alleviation of stress following administration of the anti-hyperglycemic and insulin sensitizer rosiglitazone (Yao et al., 2009). Thus, we speculate that diabetes might serve as a stress and trigger overstimulation of adrenal sympathetic tone leading to increased adrenal catecholamine levels. Approaches to improve insulin sensitivity and normalize hyperglycemia may help to overcome diabetes related changes in adrenal catecholamine levels.

8. Summary

The present study provides experimental evidence for type-2 diabetes associated behavioral and neurochemical deficits using *db/db* mice. The major findings of this dissertation report are:

- (1) Behavioral despair, age-dependent progression of psychosis-like symptoms and anxiolytic behavior in *db/db* mice.
- (2) Neurochemical deficits in *db/db* mouse brain such as abnormal DA metabolism patterns in the frontal cortex and amygdala that correlated with their behavioral deficits.
- (3) Insulin sensitizer-induced reversal of behavioral despair- but not psychosis-like behavior and impaired emotional learning in *db/db* mice.
- (4) Restoration of the majority of abnormalities in DA metabolism in the brains of *db/db* mice to normal by long-term management of hyperglycemia and insulin resistance.
- (5) Significant elevation in the adrenal catecholamine levels in *db/db* mice compared to age-matched lean controls. Rosiglitazone treatment helped to restore it to normal.

Thus, insulin resistance and hyperglycemia in *db/db* mice may be at least part of the underlying mechanisms for some but not all type-2 diabetes associated neurobehavioral deficits. Mechanisms other than those mentioned above may contribute for behavioral abnormalities such as psychosis and impaired emotional learning in *db/db* mice.

9. Prospective studies

Leptin

Leptin is an adipose tissue derived hormone. A high leptin concentration acts within the brain to signal satiety via hypothalamic LRb and boosts energy expenditure (Elmqvist et al., 1998; Friedman and Halaas, 1998). Conversely, a low leptin level stimulates neuronal circuits that signal appetite. Thus, leptin is a homeostatic mechanism for long-term regulation of metabolism and body weight (Ahima et al., 1996). *db/db* mice harbor a point mutation in the gene encoding the functional isoform (LRb) of the leptin receptor. Leptin has a profound impact on insulin resistance and glycemic control. Evidence suggests that leptin can restore normal glucose levels (Pellemounter et al., 1995) and improve insulin-sensitivity of target organs (Levin et al., 1996; Lin et al., 2002). In contrast, long-term hyperglycemia lowers leptin levels (Moriya et al., 1999). Thus leptin is a vital player for regulation of glucose homeostasis. There is widespread expression of LRb isoform receptors in the brain (Fei et al., 1997; Figlewicz et al., 2003; Mercer et al., 1996). To date, the hypothalamus was considered as the anatomical structure required for leptin signaling in the brain. Recent evidence also implicates the hippocampus, cortex and amygdala with dopaminergic innervations as crucial for leptin mediated brain functions. There is a growing perception of involvement of leptin in brain disorders. Pharmacological and pathological manipulation of endogenous plasma and brain leptin concentration is reported in many neuropsychiatric disorders and after treatment with several drug classes that can affect brain functions (Asakawa et al., 2003; Baptista and Beaulieu, 2001; Farr et al., 2006; Lu et al., 2006). Lower brain weights, reduced cortical volume and decrease in total glial and neuronal protein expressions were reported in leptin-deficient *ob/ob* and leptin-resistant *db/db* mice (Ahima et al., 1999). Recently, altered

hippocampal neuronal dendrite morphology was reported in leptin-resistant *db/db* mice (Stranahan et al., 2009). Leptin administration to leptin-deficient patients has been reported to increase concentration of gray matter (Matochik et al., 2005). Neurotrophic action of leptin was demonstrated by establishment of new neuronal circuits post-leptin treatment (Cottrell et al., 2010). Thus, leptin can have profound impact on the proliferation, maintenance and differentiation of neuronal and glial cells (Paz-Filho et al., 2008). *db/db* mice are resistant to leptin treatment because of mutation in its receptor. *ob/ob* mice are not refractory to leptin treatment and could be the important experimental tool to investigate for the possible involvement of leptin in diabetes related comorbid neurobehavioral complications. Leptin also modulates central dopaminergic neurotransmission. Overlapping leptin and DA brain circuitries play significant roles in type-2 diabetes as well as major neuropsychological disorders (Abizaïd et al., 2006; Fulton et al., 2006; Gainetdinov, 2007; Moncrieff, 2009). Leptin receptors are colocalized with DAergic neurons in vital brain loci thereby regulating major DAergic pathways (Abizaïd et al., 2006; Fulton et al., 2006). Reduced DA release and fall in TH concentration in nucleus accumbens in *ob/ob* mice can be rescued by leptin treatment (Fulton et al., 2006). Thus, leptin deficiency may contribute to some type-2 diabetes related behavioral and neurochemical deficits.

Bromocriptine

Diminished dopaminergic tone in hypothalamic neuronal circuits was reported to induce insulin resistance (Luo et al., 1997; Pijl, 2003). Type-2 diabetic Zucker *fa/fa* rats are reported to have diminished D₂R expression in the hypothalamus (Fetissov et al., 2002) with diminution of DA levels and impaired post-synaptic DA action (Meguid et al., 2000).

Further, D₂R knockout mice experience impaired insulin response to glucose, high fasting glucose and glucose intolerance (Garcia-Tornadu et al., 2010a). Interestingly, activation of D₂Rs in the *ob/ob* mice helped to lower diabetes-related metabolic abnormalities (Cincotta et al., 1997). Recently, the FDA approved D₂R agonist, bromocriptine for treatment of type-2 diabetes (de Leeuw van Weenen JE et al., 2010; Gaziano et al., 2010; Scranton and Cincotta, 2010). Bromocriptine can normalize hyperglycemia, glucose-stimulated insulin secretion (de Leeuw van Weenen JE et al., 2010; Liang et al., 1998; Scislawski et al., 1999) and hyperleptinemia (Kok et al., 2006). It also augments the antidepressant effects of selective serotonin reuptake inhibitors (Renard et al., 2001). To understand role of D₂Rs in type-2 diabetes related behavioral deficits, effect of bromocriptine should be studied in *db/db* and *ob/ob* mice.

5-HT_{2C} receptor agonist

Several studies also suggest involvement of serotonin (5-HT) in type-2 diabetes (Collin et al., 2000; Trento et al., 2010). Diminished 5-HT activity is the important attribute of depression, psychosis and anxiety. 5-HT deficiency also contributes to impaired insulin secretion and glucose intolerance (Paulmann et al., 2009). While 5-HT_{2C} receptor knockout mice experience insulin resistance and hyperglycemia (Xu et al., 2010), 5-HT_{2C} receptor agonists improve glucose homeostasis in type-2 diabetic mice (Zhou et al., 2007). Moreover, mutation of the 5-HT_{2C} receptor gene in leptin deficient *ob/ob* mice resulted in a synergistic worsening of glucose homeostasis (Wade et al., 2008). 5-HT_{2C} receptor manipulations are also been considered as a novel approach in the treatment of brain disorders like schizophrenia and depression (Cryan and Lucki, 2000; Klempin et al., 2010; Rosenzweig-Lipson et al., 2007).

These findings indicate a close integration between 5-HT-leptin system and impaired 5-HT signaling may also contribute to type-2 diabetes and related neurobehavioral complications. Thus, restoring 5-HT neurotransmission using 5-HT_{2C} receptor agonist such as CP 809101 (Siuciak et al., 2007) may help to reverse some of type-2 diabetes related neurobehavioral complications.

Neprilysin

Neprilysin (NEP) is a zinc-dependent and membrane bound neutral endopeptidase. Recently, NEP has drawn considerable research attention because of its dual therapeutic potential in neurodegenerative disorders like Alzheimer's disease (AD) (Hafez et al., 2011) and metabolic disorders such as diabetes (Liu et al., 2011; Zraika et al., 2007). The primary function of NEP is to biotransform regulatory peptides like enkephalins, endothelins and amyloid β (A β) (Johnson et al., 1999; Skidgel and Erdos, 2004). Expression of NEP was reported in the brain (Carter et al., 2006; Yasojima et al., 2001). Knocking out NEP gene in mice elevates brain A β levels with cognitive impairments (Iwata et al., 2001; Marr et al., 2004; Walther et al., 2009). On the contrary, NEP over-expression and injection of NEP reported to decrease brain A β levels with improved memory functions (El-Amouri et al., 2008; Poirier et al., 2006). Further, NEP deficiency has been shown to increase aggressive behavior and induce hypolocomotive behavior in mice. Thus, evaluation of role of NEP in type-2 diabetes related neurobehavioral functions may help to better understand underlying mechanisms.

10. Figures and tables

Table 1. Metabolic parameters of *db/db* and age-matched lean control mice

Mouse strain	Lean control	<i>db/db</i>	Lean	<i>db/db</i>
Age (weeks)	5	5	10	10
Group size (n)	18-20	12-13	18-20	12-13
Body weight (g)	19.63 ± 0.28	24.14 ± 0.6*	25.18 ± 0.56	35.97 ± 1.2*
Blood glucose (mg/dL)	145.25 ± 4.7	230.38 ± 13.25*	140.32 ± 8.81	583.20 ± 35.86*
Plasma insulin	ND	ND	1.27 ± 0.21	4.61 ± 1.52*
Food intake	4.17 ± 0.13	7.43 ± 0.43*	3.85 ± 0.11	6.74 ± 0.37*
Water intake	7.05 ± 0.13	15.66 ± 1.69*	9.76 ± 0.89	20.15 ± 2.39*
% Fat mass	14.06 ± 1.55	42.43 ± 1.63*	17.94 ± 1.01	58.92 ± 0.75*
% Lean mass	85.94 ± 1.55	57.57 ± 1.63*	70.43 ± 1.01	42.78 ± 1.40*
% Body water	60.41 ± 3.94	43.84 ± 1.50*	59.62 ± 1.00	35.85 ± 1.16*

ND, not determined. * $p < 0.05$ versus age-matched lean controls, unpaired t-test.

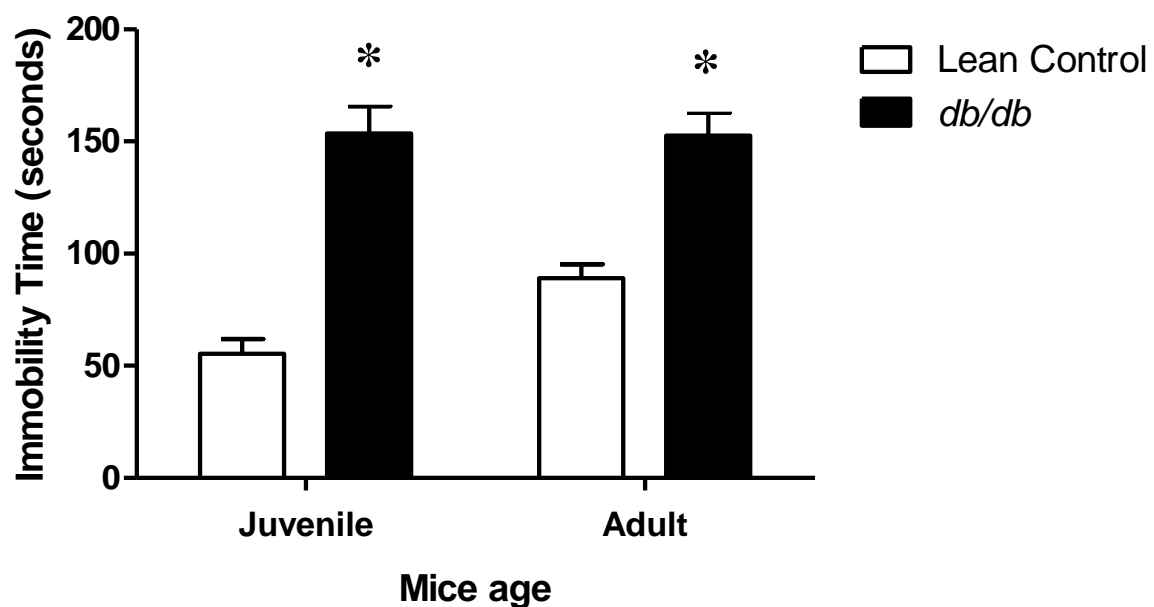
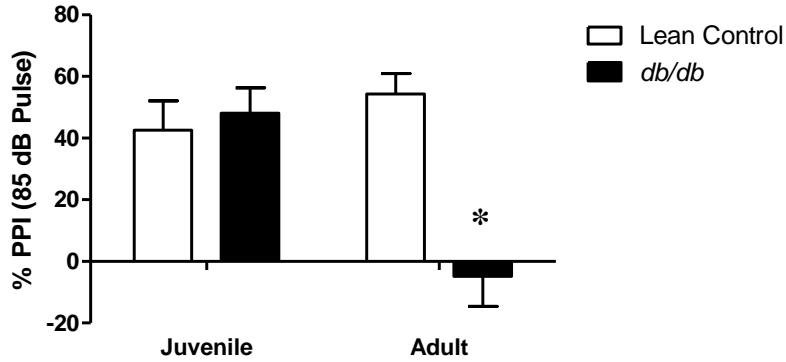


Fig. 1. Depression-like behavior of *db/db* mice. Separate groups of *juvenile* (5–6 weeks) and *adult* (10–11 weeks) *db/db* mice and their age-matched lean controls were tested for duration of immobility in forced swim test. Each mouse was forced to swim in water cylinder and scored for duration of immobility. Each bar represents means \pm S.E.M. of data from 7–10 mice per group. * $p < 0.05$ vs. age-matched lean control mice.

a)



b)

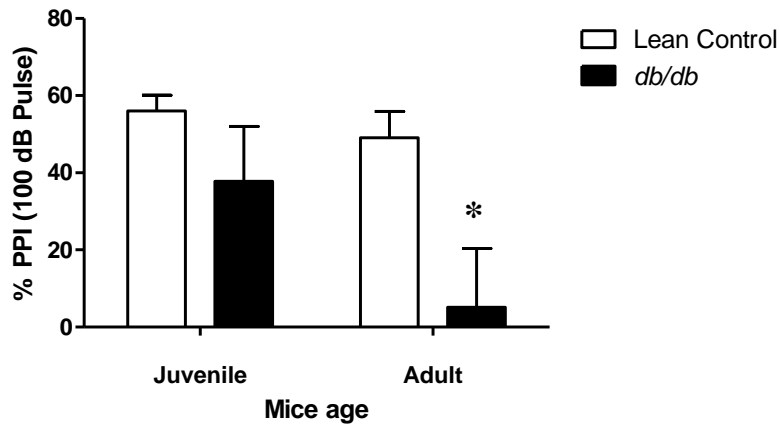
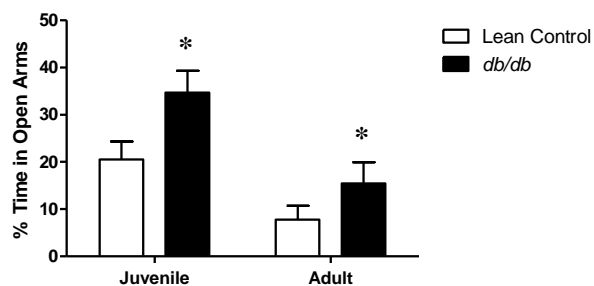
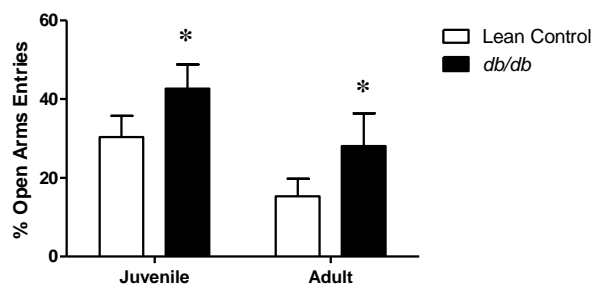


Fig. 2. Psychosis-like behavior of *db/db* mice. Different groups of *juvenile* (5–6 weeks) and *adult* (10–11 weeks) *db/db* mice and their age-matched lean controls were tested for PPI behavior. Each mouse was subjected to 5 types of trials and each trial was presented 10 times as pulse alone (85 dB or 100 dB white noise), pre-pulse alone (70 dB) and pre-pulse + pulse (70 dB + 85 dB or 70 dB + 100 dB) in randomized fashion (total trials: $5 \times 10 = 50$) with background noise (60 dB) in startle chambers and their startle responses to these stimuli (in Newtons; Max[N]) were recorded using pressure-sensitive plates. Each bar represents means \pm S.E.M. of data from 7–10 mice per group. * $p < 0.05$ vs. age-matched lean control mice.

a)



b)



c)

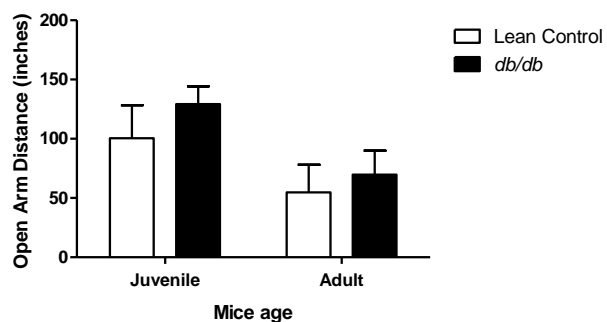
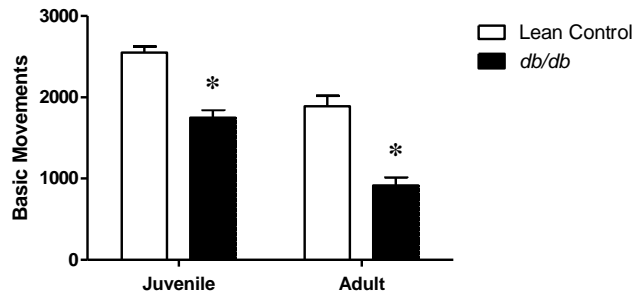
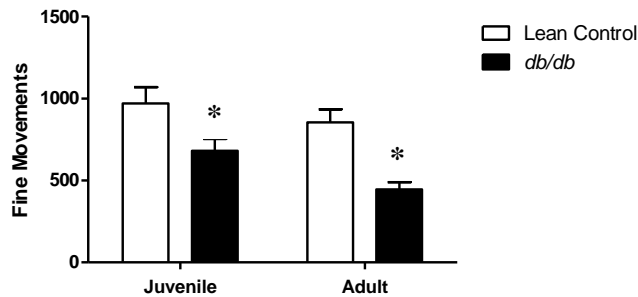


Fig. 3. Anxiolytic behavior of *db/db* mice showing a) Percent time spent in open arms b) Percent open arms entries and c) Open arms distance traveled (inches) on elevated plus maze. Different groups of *juvenile* (5–6 weeks) and *adult* (10–11 weeks) *db/db* mice and their age-matched lean littermates were subjected to 5 min elevated plus maze test. Each bar represents means \pm S.E.M. of data from 7–10 mice per group. * $p < 0.05$ vs. age-matched lean control mice.

a)



b)



c)

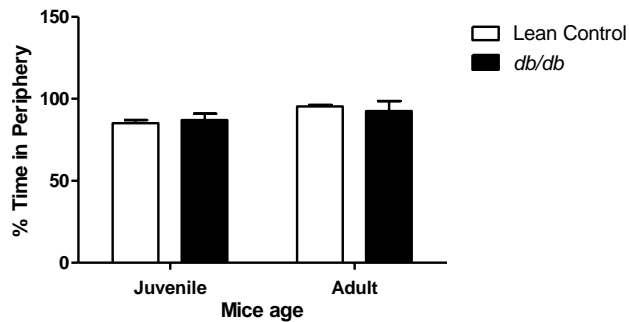
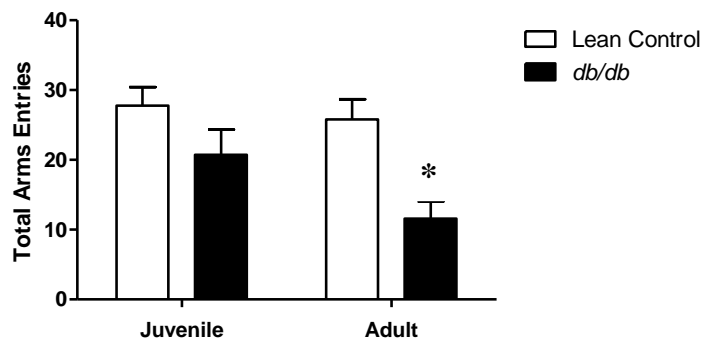
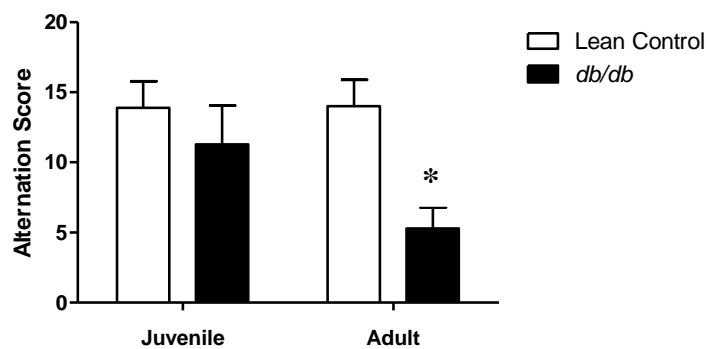


Fig. 4. Hypo-locomotive and thigmotaxis behavior of *db/db* mice in open field test showing a) Basic movements b) Fine movements and c) Percent time spent in periphery. Different groups of *juvenile* (5–6 weeks) and *adult* (10–11 weeks) *db/db* mice and their age-matched lean controls were subjected to 10 min open field test. Each bar represents means \pm S.E.M. of data from 7–10 mice per group. * $p < 0.05$ vs. age-matched lean control mice.

a)



b)



c)

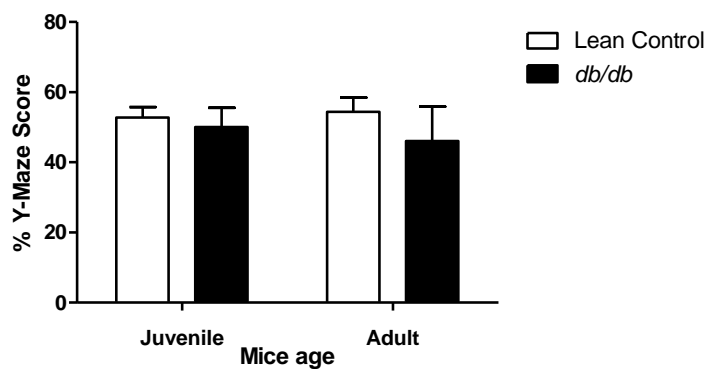


Fig. 5. Working memory test in *db/db* mice. Different groups of *juvenile* (5–6 weeks) and *adult* (10–11 weeks) *db/db* mice and their age-matched lean controls were subjected to 8 min Y-maze test and scored for a) total arm entries, b) alternations and c) percent Y-maze scores. Each bar represents means \pm S.E.M. of data from 7–10 mice per group. * $p < 0.05$ vs. age-matched lean control mice.

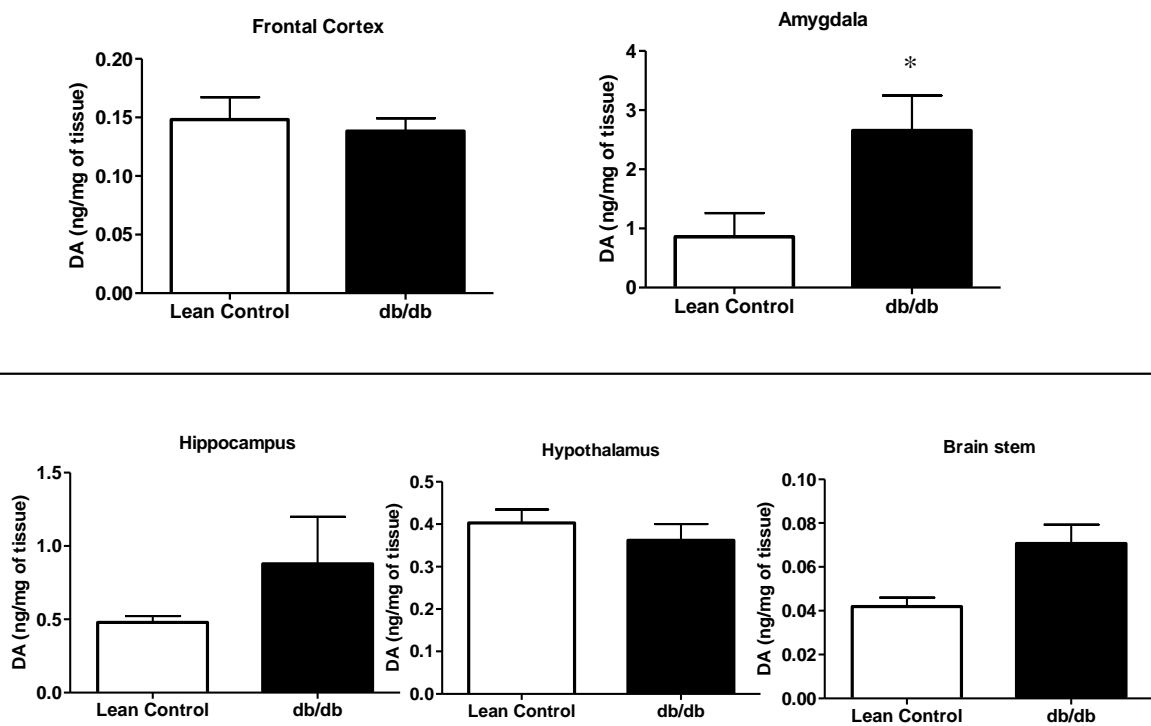


Fig. 6. Dopamine (DA) concentrations in distinct brain regions of *db/db* mice. Frontal cortex, amygdala, hippocampus, hypothalamus and brainstem of 11-12 week old *db/db* mice and age-matched lean controls were evaluated for DA concentrations by reverse-phase HPLC. Each bar represents means \pm S.E.M. of data from 7-10 mice per group. * $p < 0.05$ vs. age-matched lean control mice; non-parametric unpaired t-test with Welch's correction.

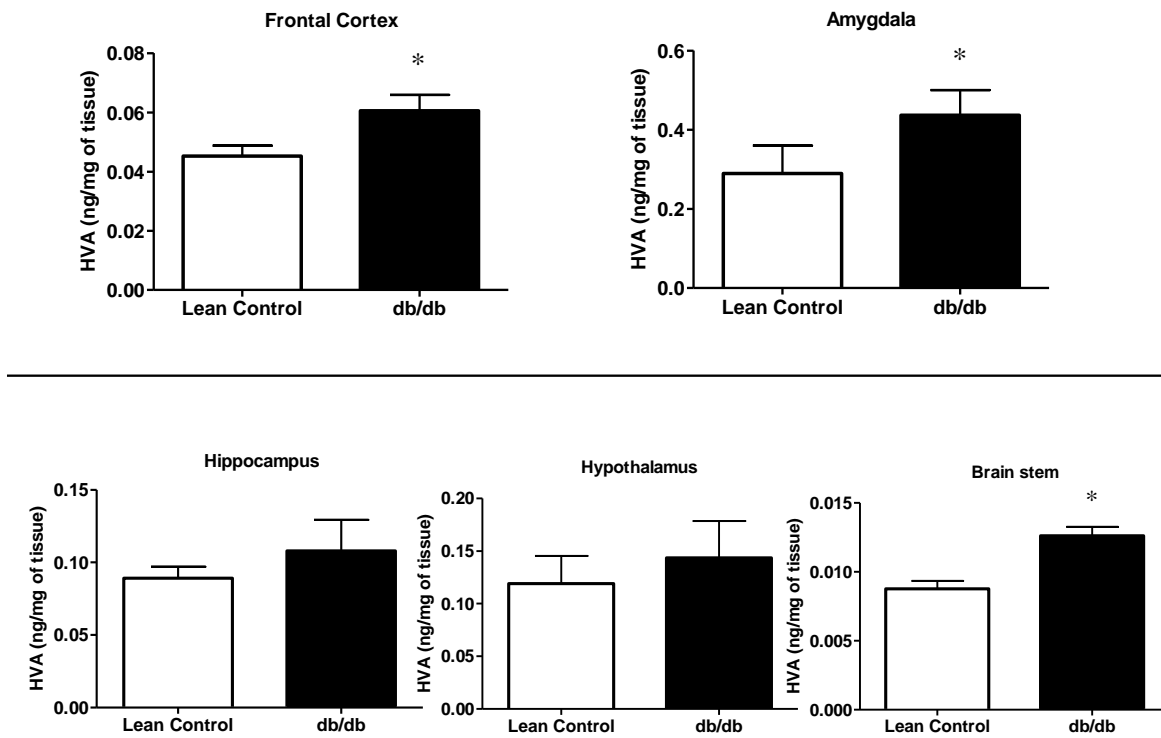


Fig. 7. Homovanillic acid (HVA) concentrations in distinct brain regions of *db/db* mice. Frontal cortex, amygdala, hippocampus, hypothalamus and brainstem of 11-12 week old *db/db* mice and age-matched lean controls were evaluated for HVA concentrations by reverse-phase HPLC. Each bar represents means \pm S.E.M. of data from 7-10 mice per group. * $p < 0.05$ vs. age-matched lean control mice; non-parametric unpaired t-test with Welch's correction.

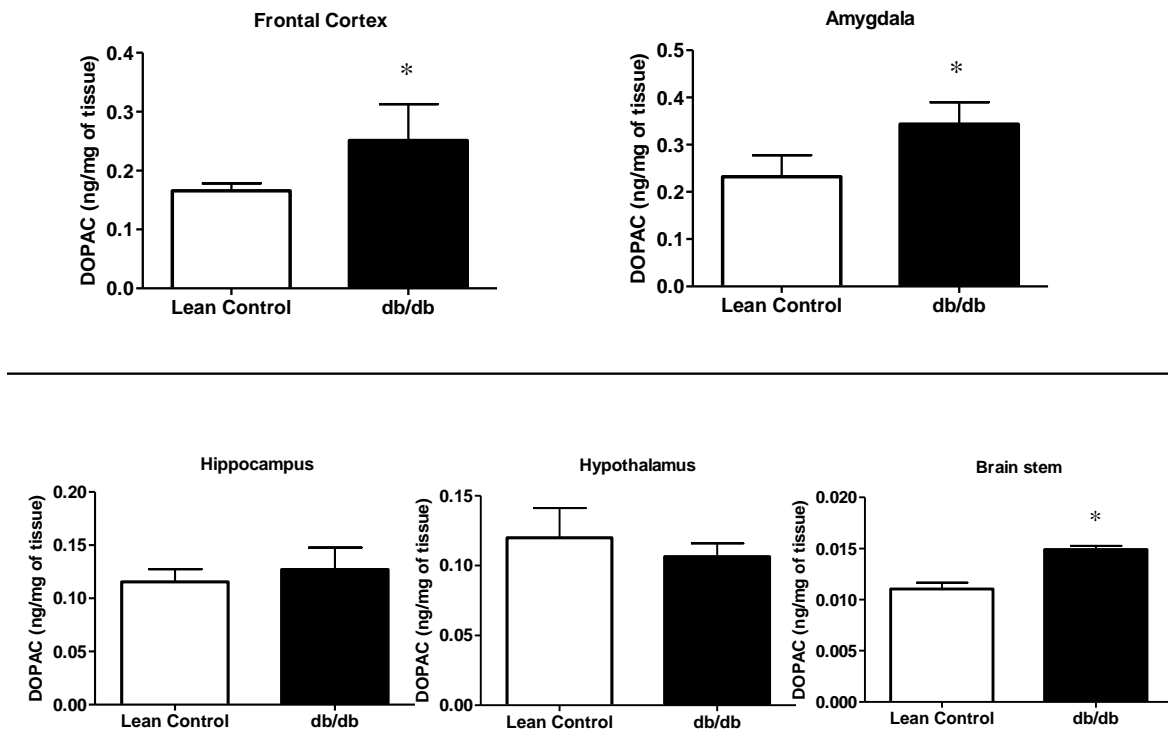


Fig. 8. Dihydroxyphenylacetic acid (DOPAC) concentrations in distinct brain regions of *db/db* mice. Frontal cortex, amygdala, hippocampus, hypothalamus and brainstem of 11-12 week old *db/db* mice and age-matched lean controls were evaluated for DOPAC concentrations by reverse-phase HPLC. Each bar represents means \pm S.E.M. of data from 7-10 mice per group. * $p < 0.05$ vs. age-matched lean control mice; non-parametric unpaired t-test with Welch's correction.

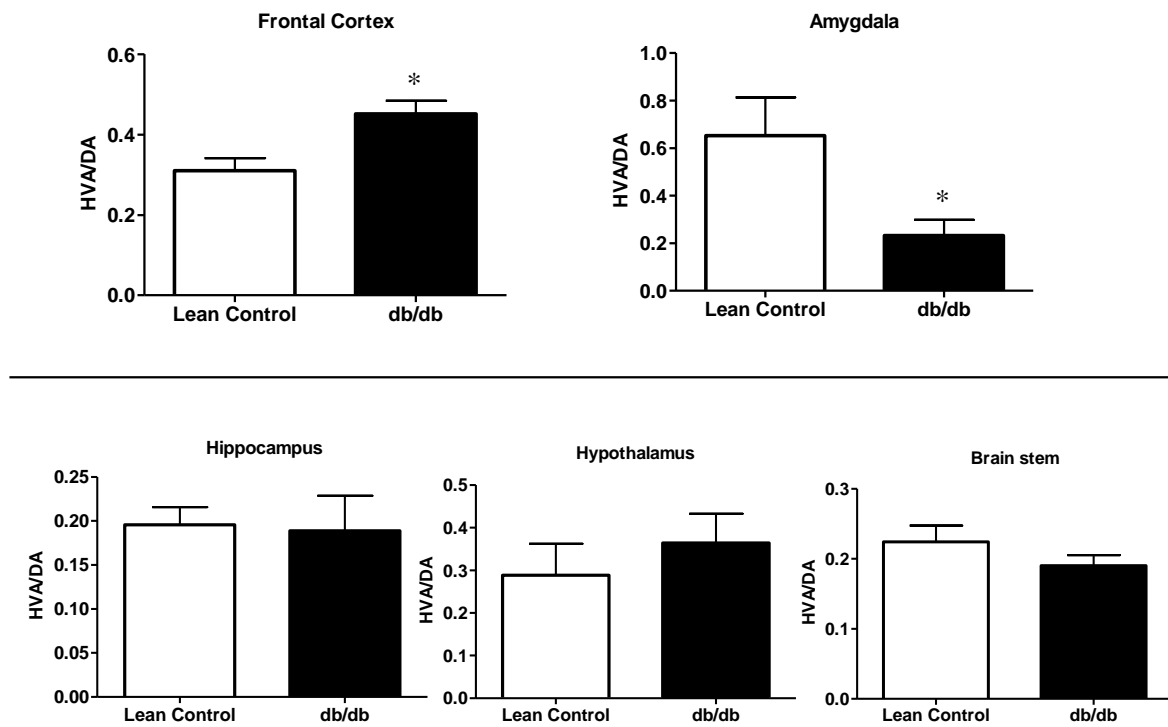


Fig. 9. HVA/DA ratios in distinct brain regions of *db/db* mice. Frontal cortex, amygdala, hippocampus, hypothalamus and brainstem of 11-12 week old *db/db* mice and age-matched lean controls were evaluated for DA and HVA concentrations. HVA/DA ratios were calculated as an index of DA usage. Each bar represents means \pm S.E.M. of data from 7-10 mice per group. * $p < 0.05$ vs. age-matched lean control mice; non-parametric unpaired t-test with Welch's correction.

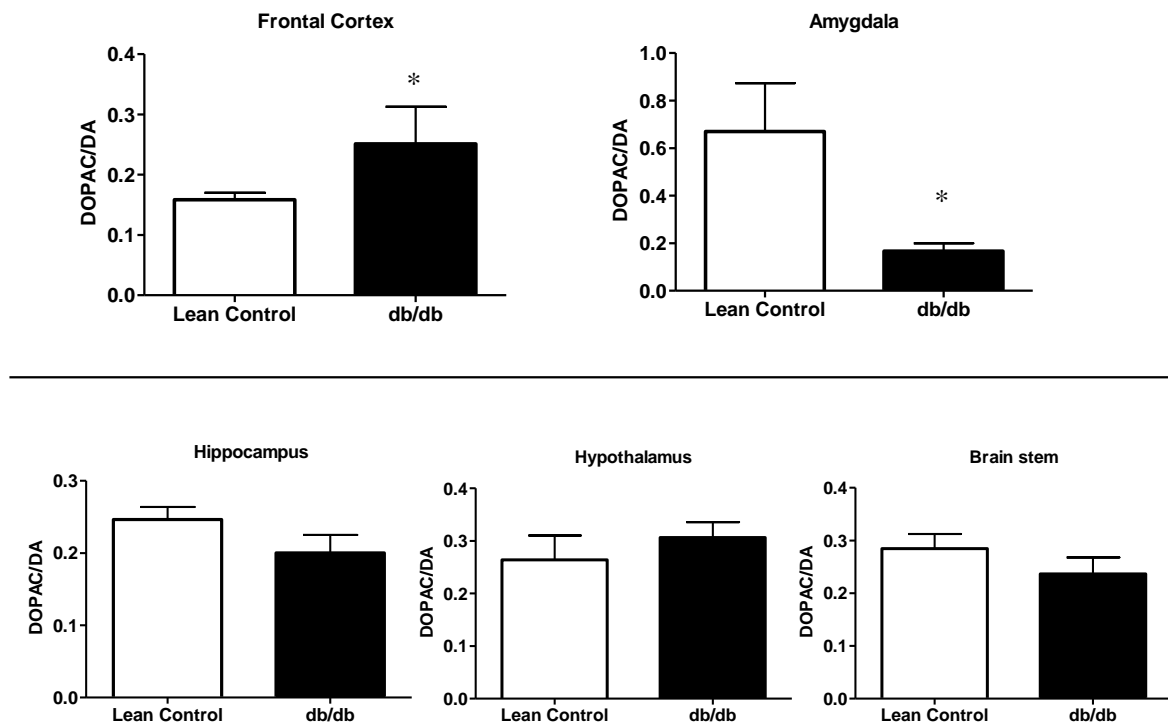


Fig. 10. DOPAC/DA ratios in distinct brain regions of *db/db* mice. Frontal cortex, amygdala, hippocampus, hypothalamus and brainstem of 11-12 week old *db/db* mice and age-matched lean controls were evaluated for DA and DOPAC concentrations. DOPAC/DA ratios were calculated as an index of DA usage. Each bar represents means \pm S.E.M. of data from 7-10 mice per group. * $p < 0.05$ vs. age-matched lean control mice; non-parametric unpaired t-test with Welch's correction.

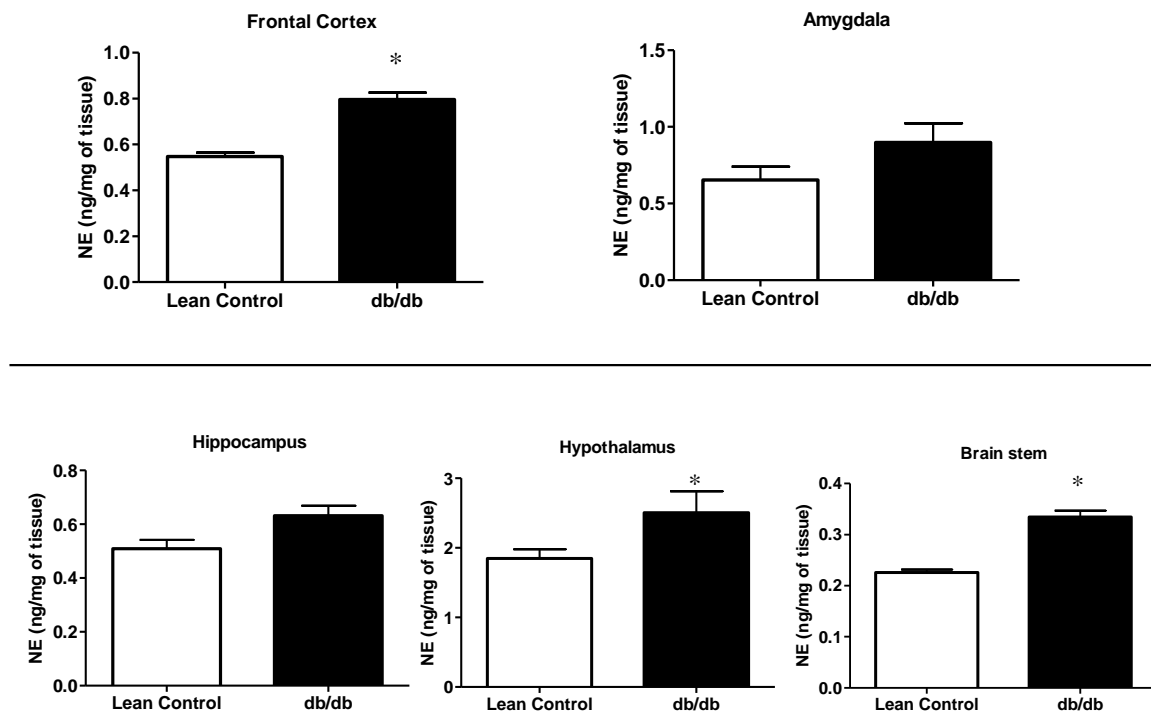


Fig. 11. Norepinephrine (NE) concentrations in distinct brain regions of *db/db* mice. Frontal cortex, amygdala, hippocampus, hypothalamus and brainstem of 11-12 week old *db/db* mice and age-matched lean controls were evaluated for NE concentrations by reverse-phase HPLC. Each bar represents means \pm S.E.M. of data from 7-10 mice per group. * $p < 0.05$ vs. age-matched lean control mice; non-parametric unpaired t-test with Welch's correction.

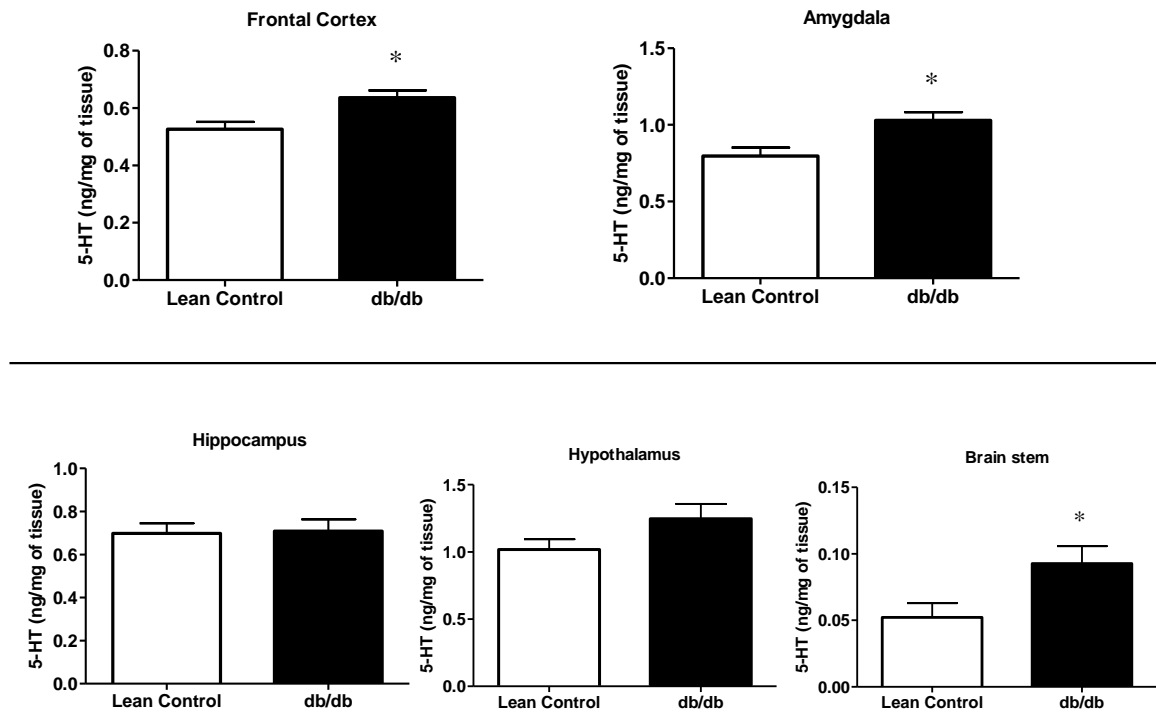


Fig. 12. 5-Hydroxytryptamine (5-HT) concentrations in distinct brain regions of *db/db* mice. Frontal cortex, amygdala, hippocampus, hypothalamus and brainstem of 11-12 week old *db/db* mice and age-matched lean controls were evaluated for 5-HT concentrations by reverse-phase HPLC. Each bar represents means \pm S.E.M. of data from 7-10 mice per group. * $p < 0.05$ vs. age-matched lean control mice; non-parametric unpaired t-test with Welch's correction.

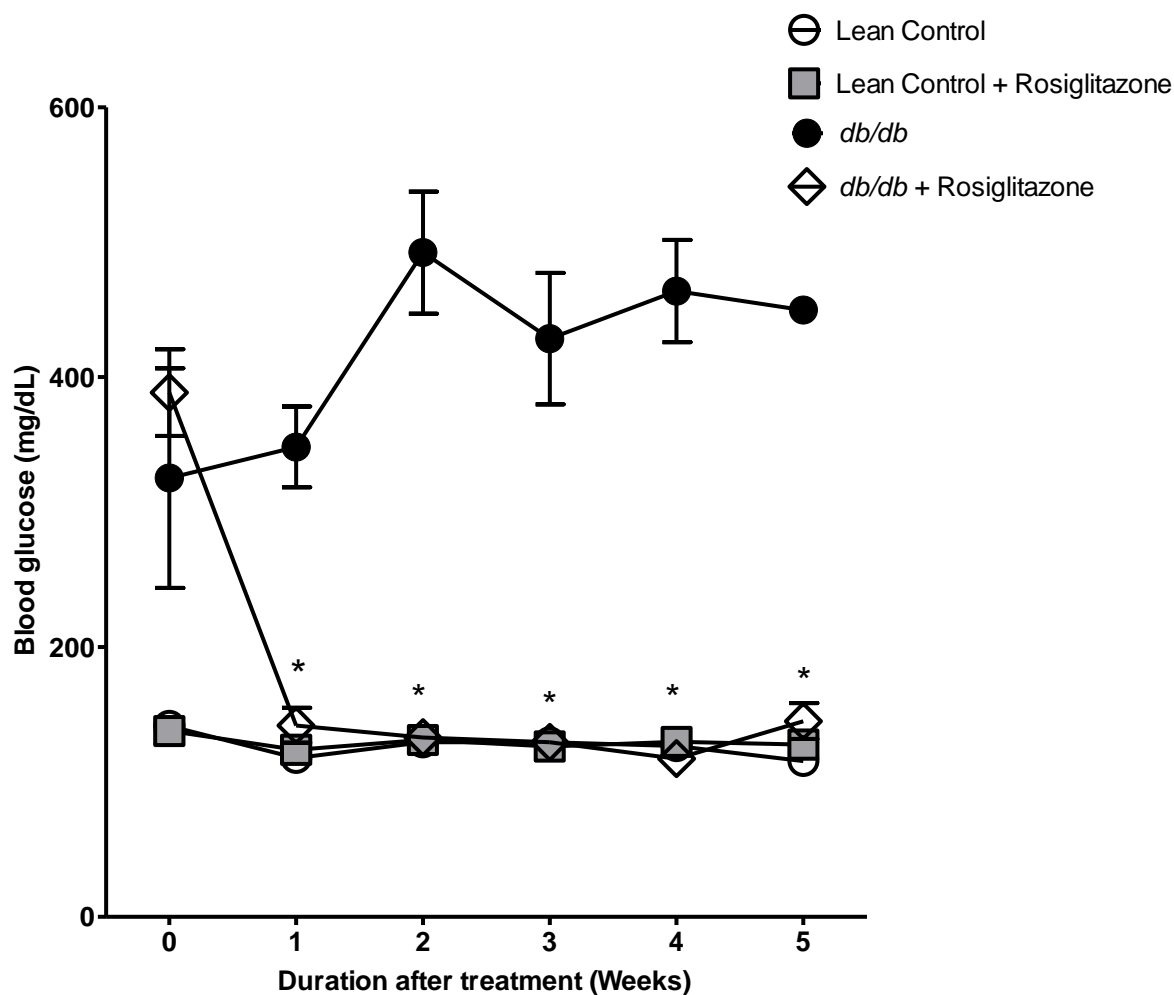


Fig. 13. Effect of rosiglitazone on blood glucose levels in lean control and *db/db* mice. 5-6 week old *db/db* mice and their age-matched lean controls were fed with either standard chow or rosiglitazone-mixed chow (71 mg/kg chow, 20 mg/kg/day p.o.) (n = 5-8) for 5 weeks and weekly monitored for blood glucose concentration. Each bar represents means \pm S.E.M. of data from 5-13 mice per group. * $p < 0.05$ vs. age-matched lean control mice.

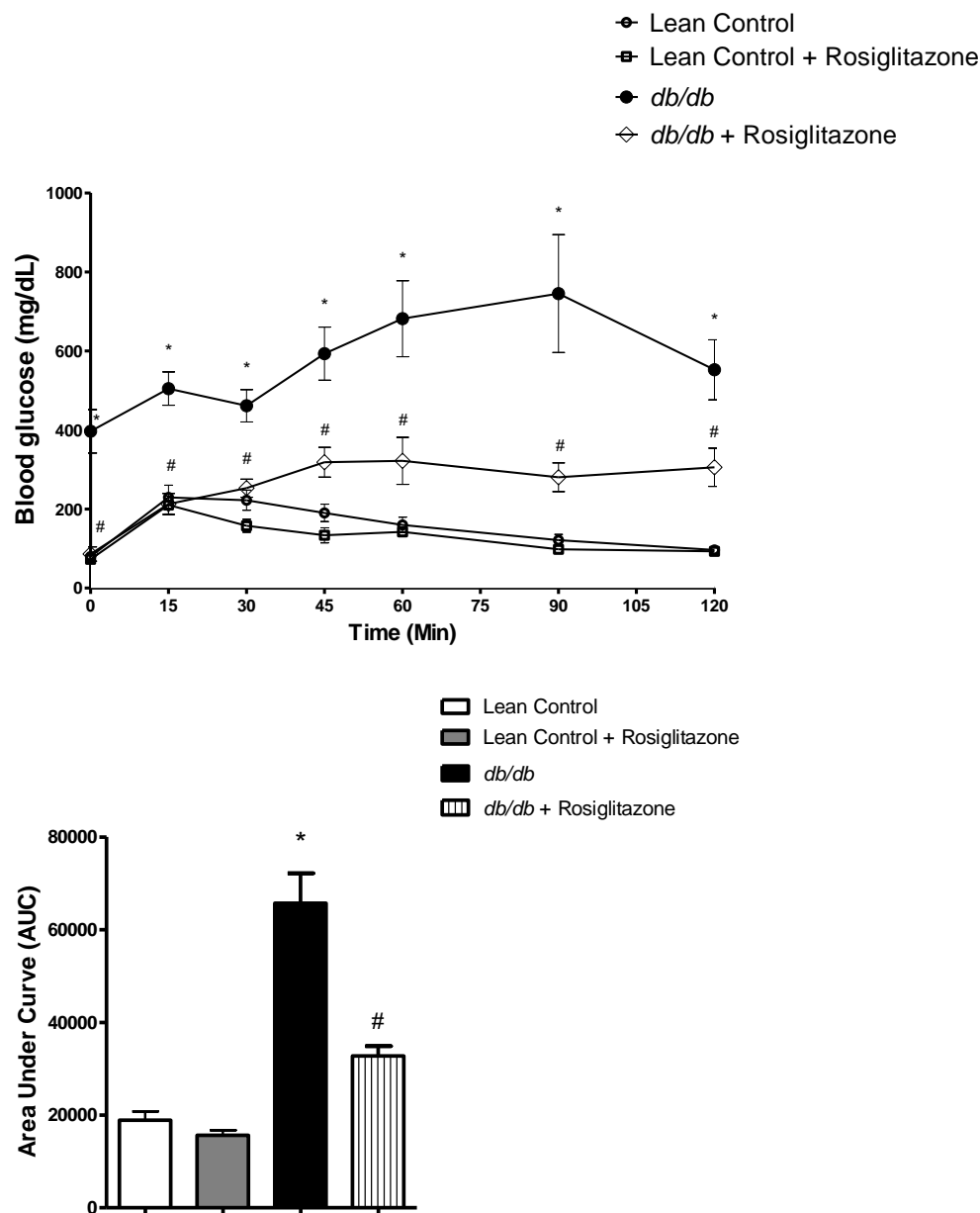


Fig. 14 Rosiglitazone treatment improved glucose utilization by *db/db* mice. Separate groups of *db/db* mice and their age-matched lean controls were administered either standard chow or rosiglitazone-mixed chow (71 mg/kg chow, 20 mg/kg/day p.o.) (n = 5-8) for 5 weeks. Mice were fasted overnight. Blood glucose concentrations were measured at 0, 15, 30, 45, 60, 90 and 120 minute post-glucose (1.5 gm/kg, i.p.) injection from the blood samples taken from cut made on tip of mouse tail. Area under the curve (AUC) was calculated to study treatment effect on mouse's glucose tolerability. Each bar represents means \pm S.E.M. of data from 5-13 mice per group. * $p < 0.05$ vs. age-matched lean control mice.

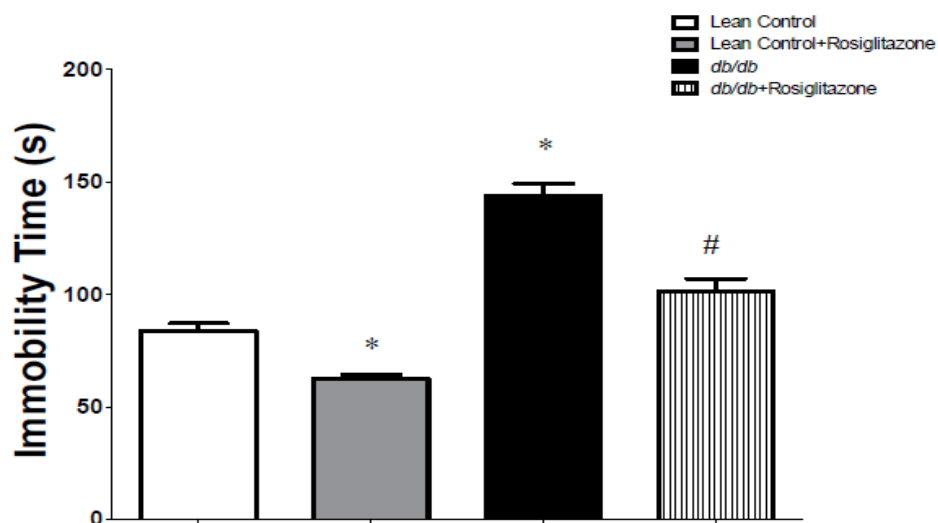


Fig. 15. Rosiglitazone reversed depression-like behavior of *db/db* mice. 5-6 week old *db/db* mice and their age-matched lean controls were fed with standard chow or rosiglitazone-mixed chow (71 mg/kg chow, 20 mg/kg/day p.o.) (n = 5-8) for 5 weeks. 10-11 week old mice were tested for duration of immobility in the forced swim test. Each mouse was forced to swim in water cylinder and scored for duration of immobility. Each bar represents means \pm S.E.M. of data from 5-8 mice per group. * $p < 0.05$ vs. age-matched lean control mice; # $p < 0.05$ vs. age-matched *db/db* mice.

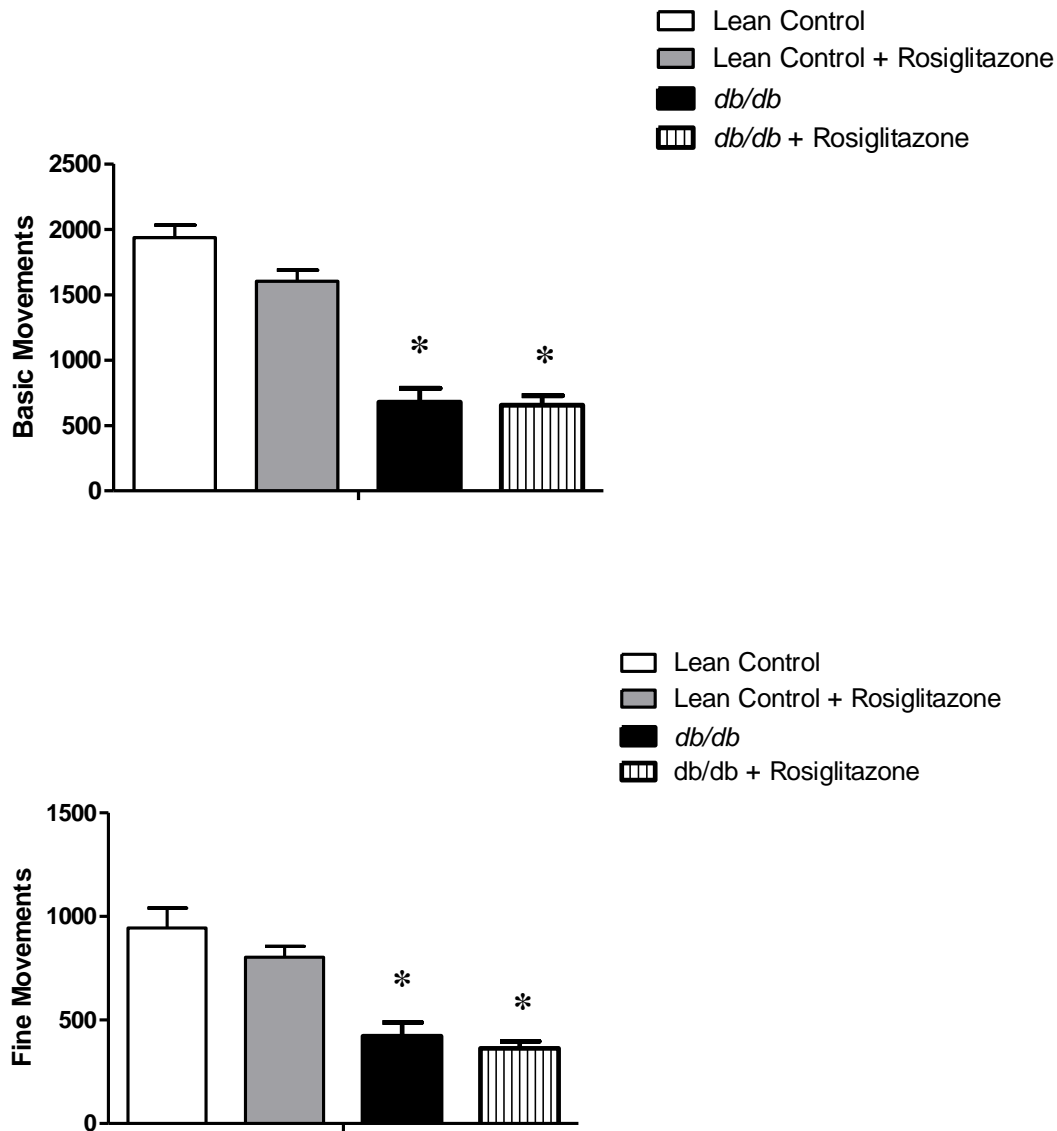


Fig. 16. Rosiglitazone treatment does not reverse hypolocomotive behavior of *db/db* mice. 5-6 week old *db/db* mice and their age-matched lean controls were fed with standard chow or Rosiglitazone-mixed chow (71 mg/kg chow, 20 mg/kg/day p.o.) (n = 5-8) for 5 weeks. 10-11 week old mice were scored for A) Basic movements and B) Fine movements in open field test. Individual mice were subjected to 10 minute open field test. Each bar represents means \pm S.E.M. of data from 5-8 mice per group. * $p < 0.05$ vs. age-matched lean control mice.

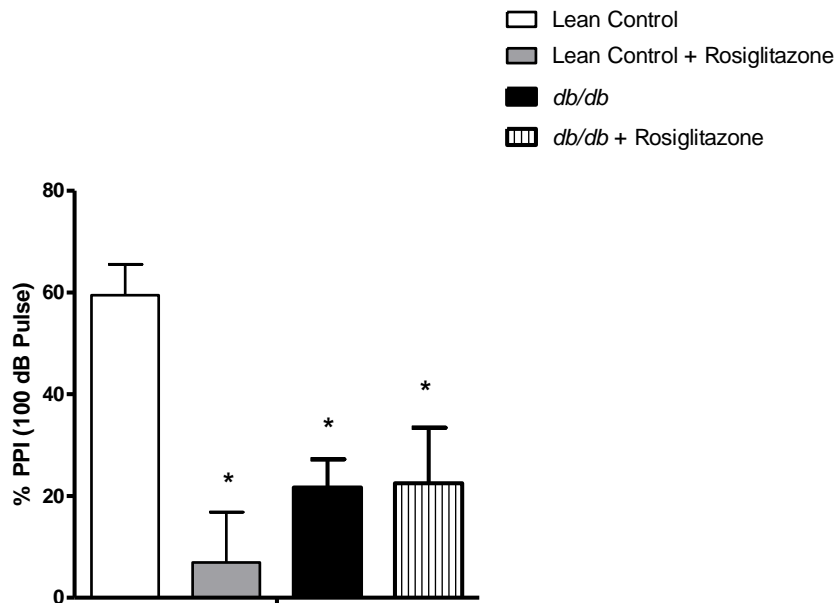
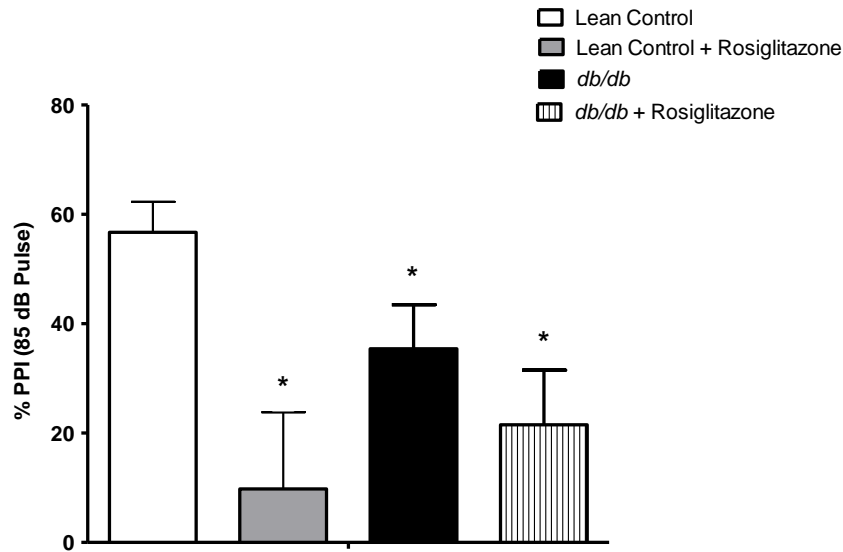


Fig. 17. Rosiglitazone treatment does not reverse psychosis-like behavior of *db/db* mice. 5-6 week old *db/db* mice and their age-matched lean controls were fed with standard chow or Rosiglitazone-mixed chow (71 mg/kg chow, 20 mg/kg/day p.o.) for 5 weeks. 10-11 week old mice were tested for PPI behavior. Each mouse was subjected to 5 types of trials and each trial was presented 10 times as pulse alone (85 dB or 100 dB white noise), pre-pulse alone (70 dB) and pre-pulse + pulse (70 dB + 85 dB or 70 dB + 100 dB) in randomized fashion (total trials: 5 x 10 = 50) in startle chambers and their startle responses (in Newtons; Max[N]) were recorded using pressure-sensitive plates. Each bar represents means \pm S.E.M. of data from 5-8 mice per group. * $p < 0.05$ vs. age-matched lean control mice.

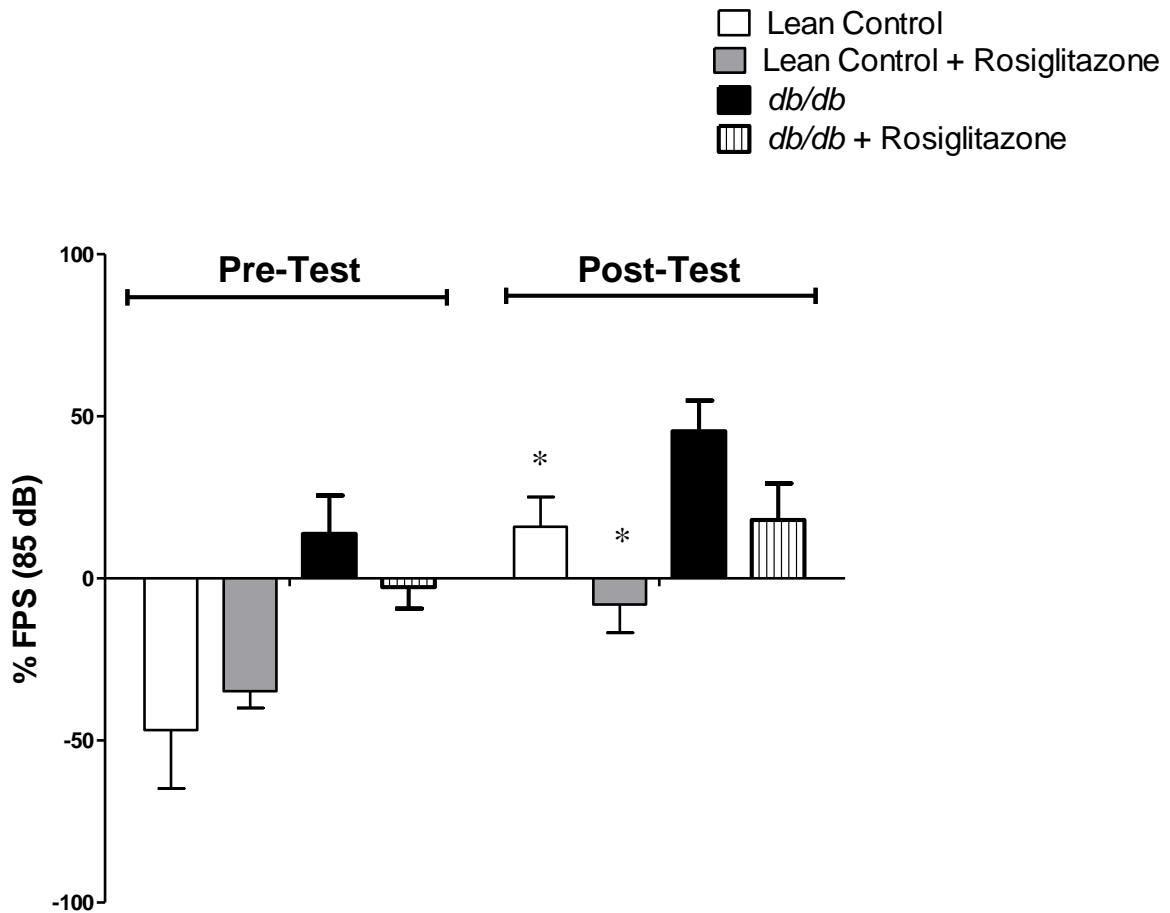


Fig. 18. Rosiglitazone does not alter *db/db* mouse behavior in the fear potentiated startle test. 5-6 week old *db/db* mice and their age-matched lean controls were fed with standard chow or Rosiglitazone-mixed chow (71 mg/kg chow, 20 mg/kg/day p.o.) (n = 5-8) for 5 weeks. 10-11 week old mice were tested for FPS behavior. During pre-test and post-test each mouse was subjected to 2 different trials (i) startle stimulus (85 dB white noise startle stimulus) and (ii) startle stimulus with tone (70dB, 30 s., 12 kHz pure tone plus 85 dB white noise) with an inter-trial interval of 1 min. Each trial was repeated 8 times and presented in randomized fashion (total = 16 trials). The % FPS responses for were calculated. The 2-day training phase preceded to post-test was consisted of 20 trials/day [70dB, 30 s., 12 kHz (conditioned stimulus, CS) tone] overlapping and terminating with a 500 ms 0.4 mA shock (unconditioned stimulus, US) began 29.5 s after the onset of the tone with inter-trial interval between 1-3 min.

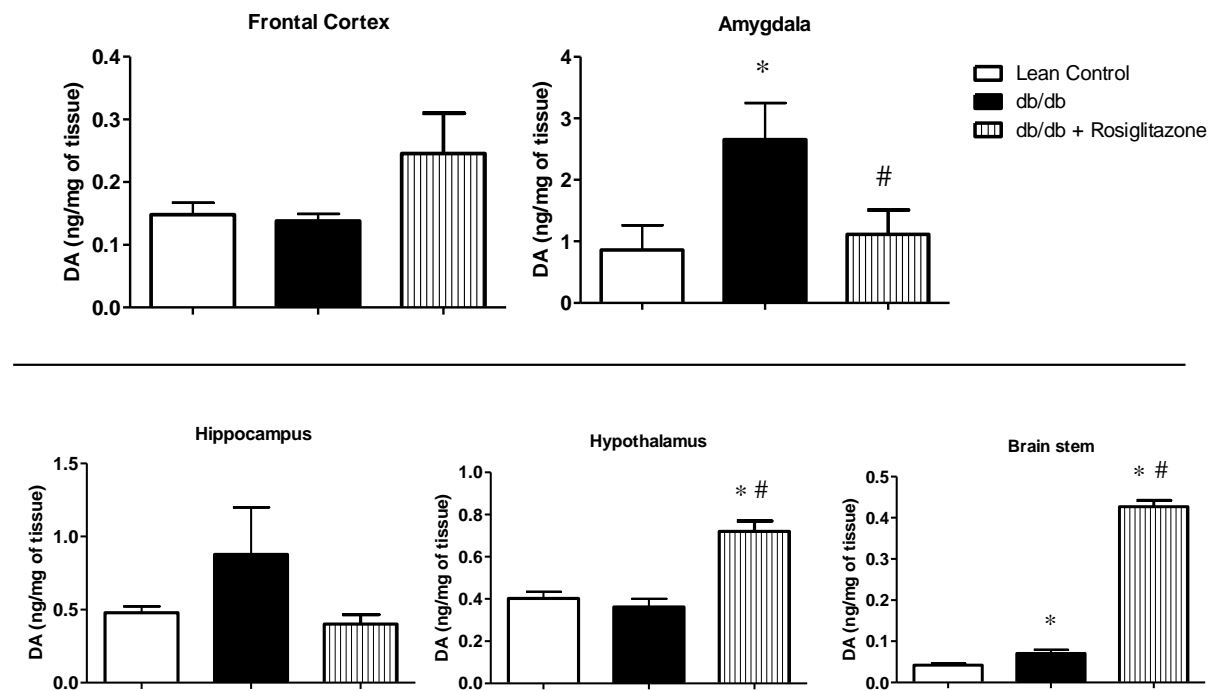


Fig. 19. Effect of rosiglitazone treatment on DA concentrations in distinct brain regions of *db/db* mice. Frontal cortex, amygdala, hippocampus, hypothalamus and brainstem of 11-12 week old *db/db* mice and age-matched lean controls were evaluated for DA concentrations by reverse-phase HPLC. Each bar represents means \pm S.E.M. of data from 7-10 mice per group. * $p < 0.05$ vs. age-matched lean control mice; # $p < 0.05$ vs. age-matched *db/db* mice.

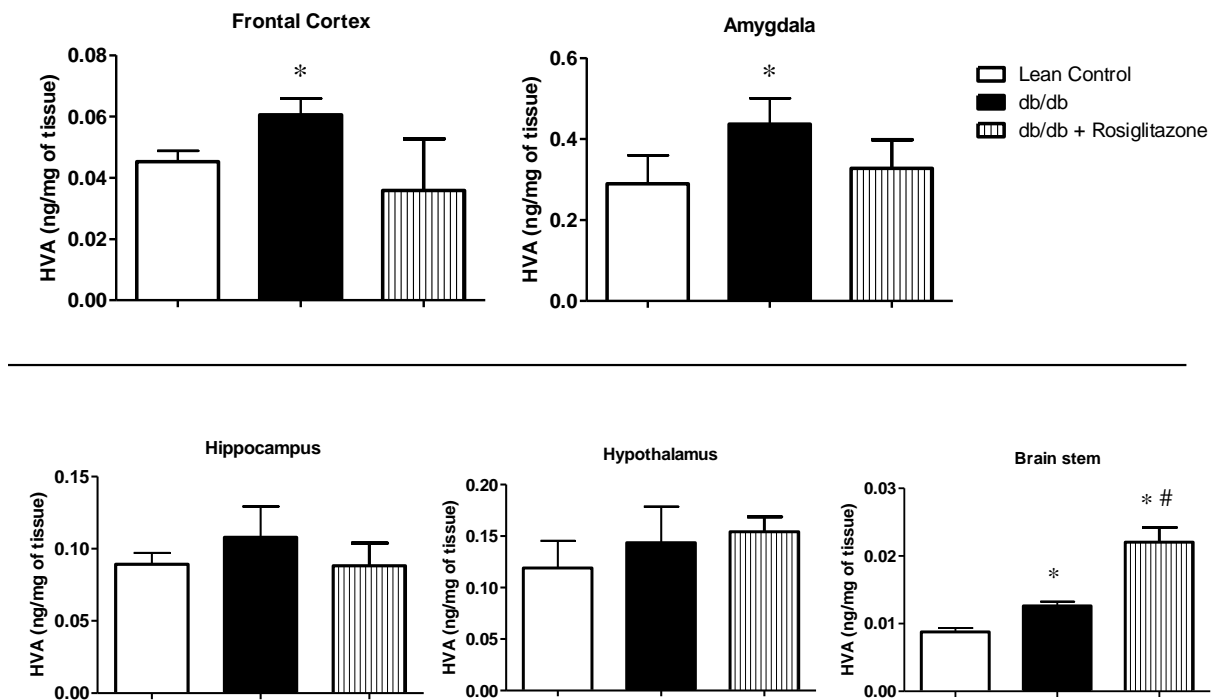


Fig. 20. Effect of rosiglitazone treatment on HVA concentrations in distinct brain regions of *db/db* mice. Frontal cortex, amygdala, hippocampus, hypothalamus and brainstem of 11-12 week old *db/db* mice and age-matched lean controls were evaluated for HVA concentrations by reverse-phase HPLC. Each bar represents means \pm S.E.M. of data from 7-10 mice per group. * $p < 0.05$ vs. age-matched lean control mice; # $p < 0.05$ vs. age-matched *db/db* mice.

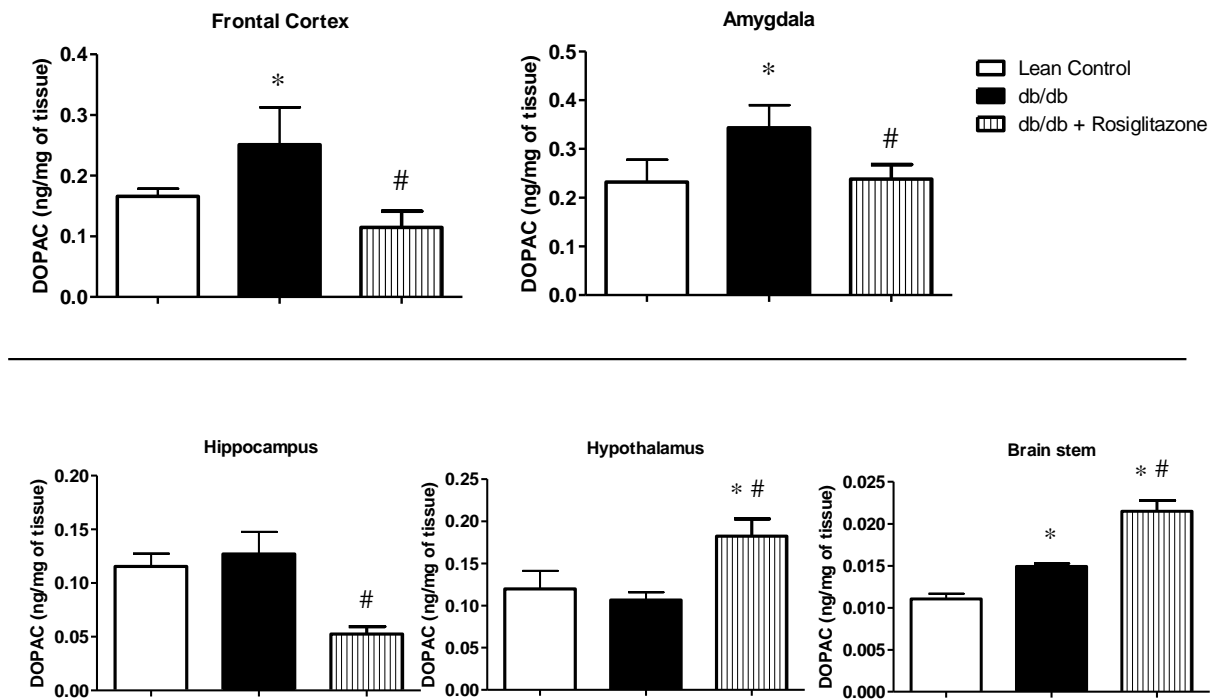


Fig. 21. Effect of rosiglitazone treatment on DOPAC concentrations in distinct brain regions of *db/db* mice. Frontal cortex, amygdala, hippocampus, hypothalamus and brainstem of 11-12 week old *db/db* mice and age-matched lean controls were evaluated for DOPAC concentrations by reverse-phase HPLC. Each bar represents means \pm S.E.M. of data from 7-10 mice per group. * $p < 0.05$ vs. age-matched lean control mice; # $p < 0.05$ vs. age-matched *db/db* mice.

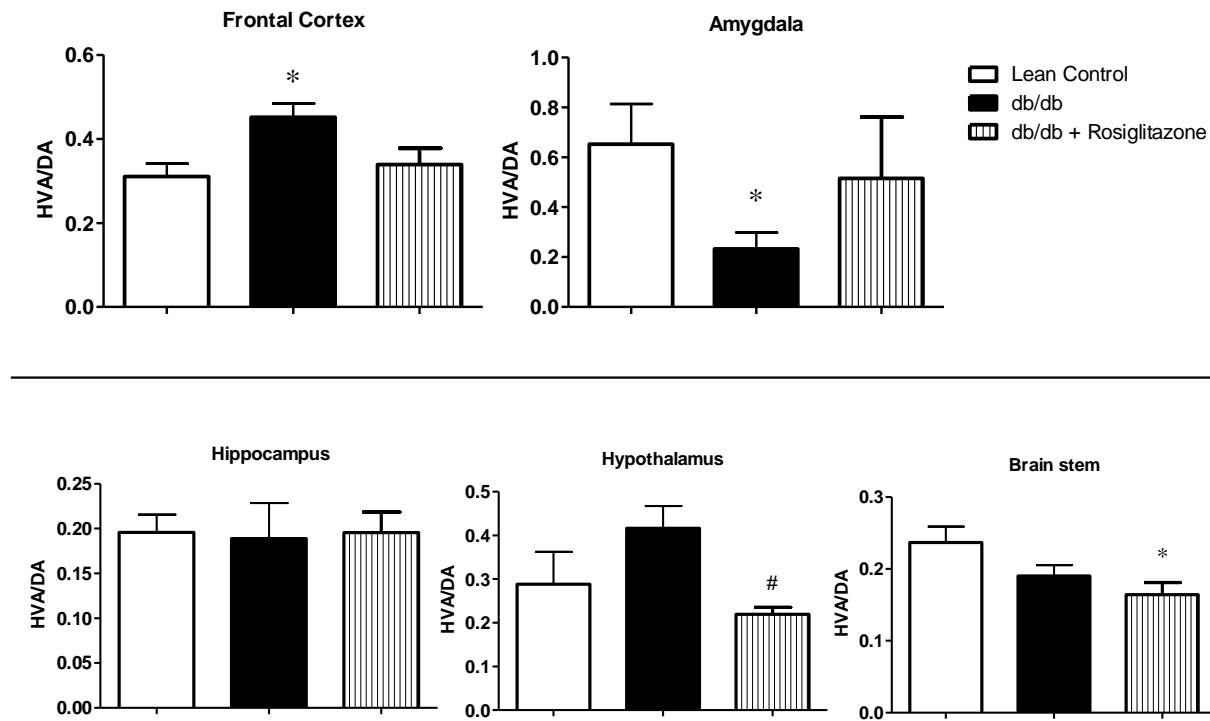


Fig. 22. Effect of rosiglitazone treatment on HVA/DA ratios in distinct brain regions of *db/db* mice. Frontal cortex, amygdala, hippocampus, hypothalamus and brainstem of 11-12 week old *db/db* mice and age-matched lean controls were evaluated for DA and HVA concentrations. HVA/DA ratios were calculated as an index of DA usage. Each bar represents means \pm S.E.M. of data from 7-10 mice per group. * $p < 0.05$ vs. age-matched lean control mice; # $p < 0.05$ vs. age-matched *db/db* mice.

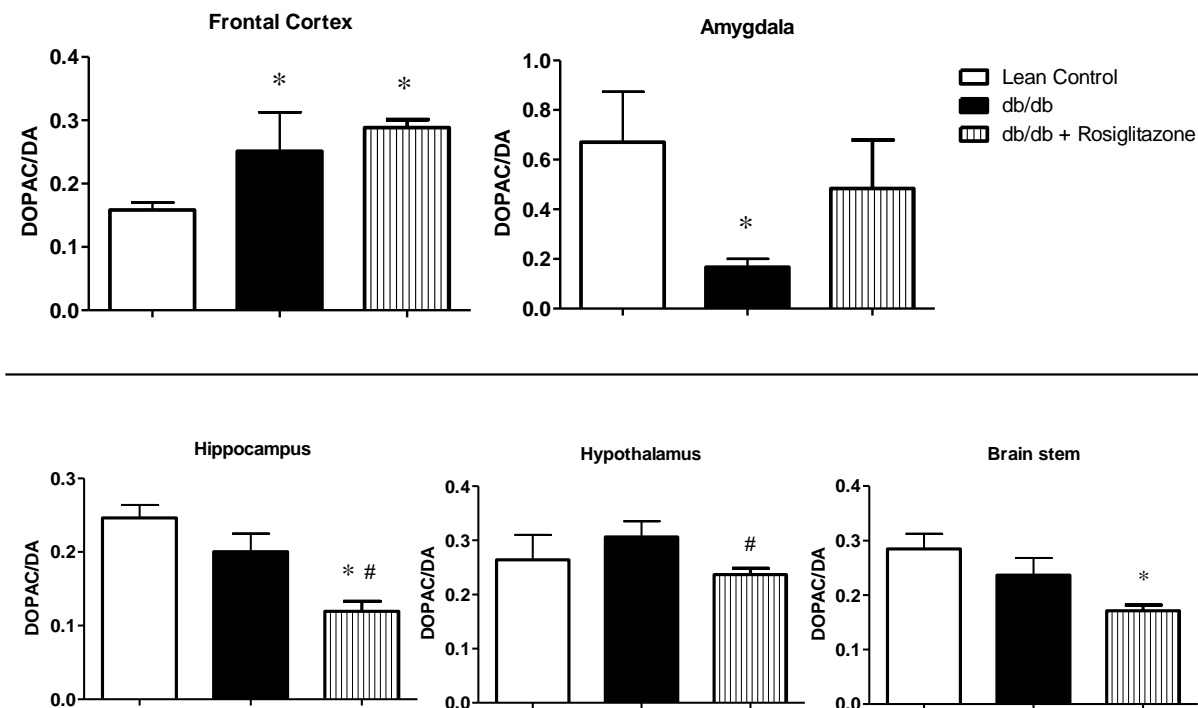


Fig. 23. Effect of rosiglitazone treatment on DOPAC/DA ratios in distinct brain regions of *db/db* mice. Frontal cortex, amygdala, hippocampus, hypothalamus and brainstem of 11-12 week old *db/db* mice and age-matched lean controls were evaluated for DA and DOPAC concentrations. DOPAC/DA ratios were calculated as an index of DA usage. Each bar represents means \pm S.E.M. of data from 7-10 mice per group. * $p < 0.05$ vs. age-matched lean control mice; # $p < 0.05$ vs. age-matched *db/db* mice.

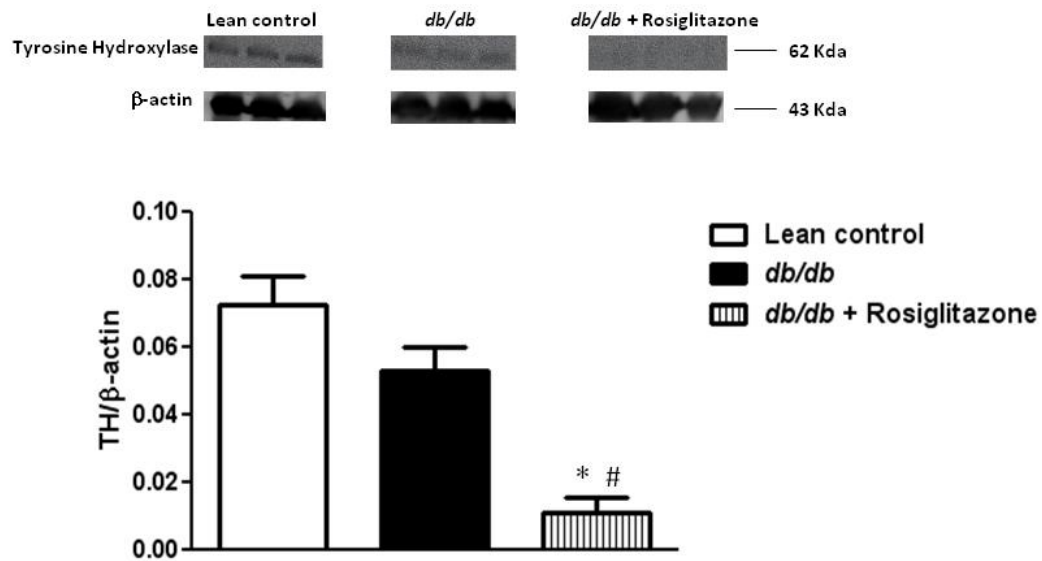


Fig. 24. Effect of rosiglitazone treatment on tyrosine hydroxylase (TH) expression in the frontal cortex of *db/db* mice. Unpaired t-test showed that there was no significant change in the TH expression in the *db/db* mice compared to age-matched lean control ($p > 0.05$). Each bar represents means \pm S.E.M. of data from $n = 3$ sample wells per group. * $p < 0.05$ vs. age-matched lean control mice; # $p < 0.05$ vs. age-matched *db/db* mice.

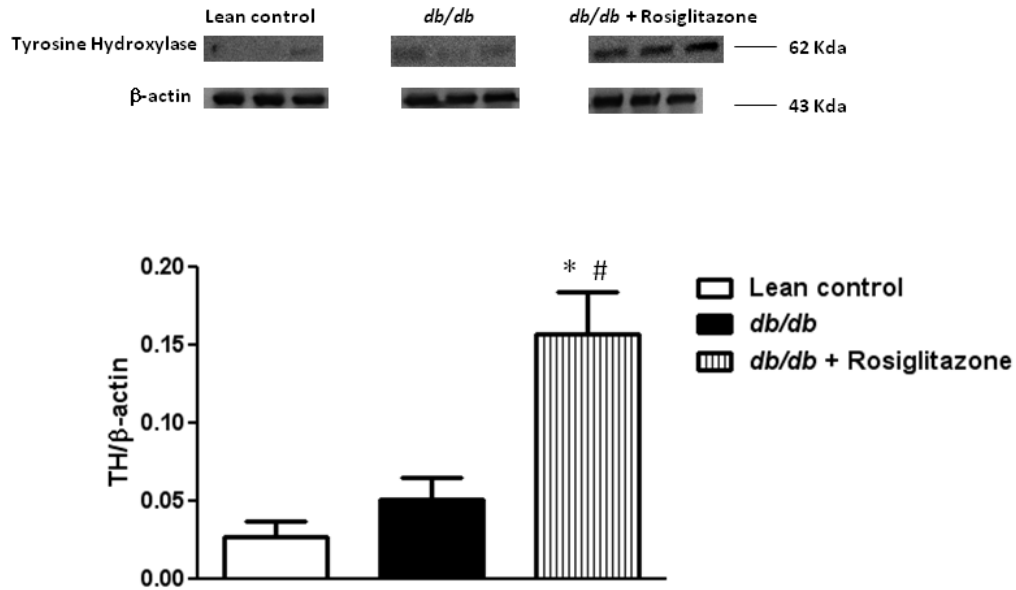


Fig. 25. Effect of rosiglitazone treatment on tyrosine hydroxylase (TH) expression in the amygdala of *db/db* mice. Unpaired t-test showed that there was no significant change in the TH expression in the *db/db* mice compared to age-matched lean control ($p > 0.05$). Each bar represents means \pm S.E.M. of data from $n = 3$ sample wells per group. * $p < 0.05$ vs. age-matched lean control mice; # $p < 0.05$ vs. age-matched *db/db* mice.

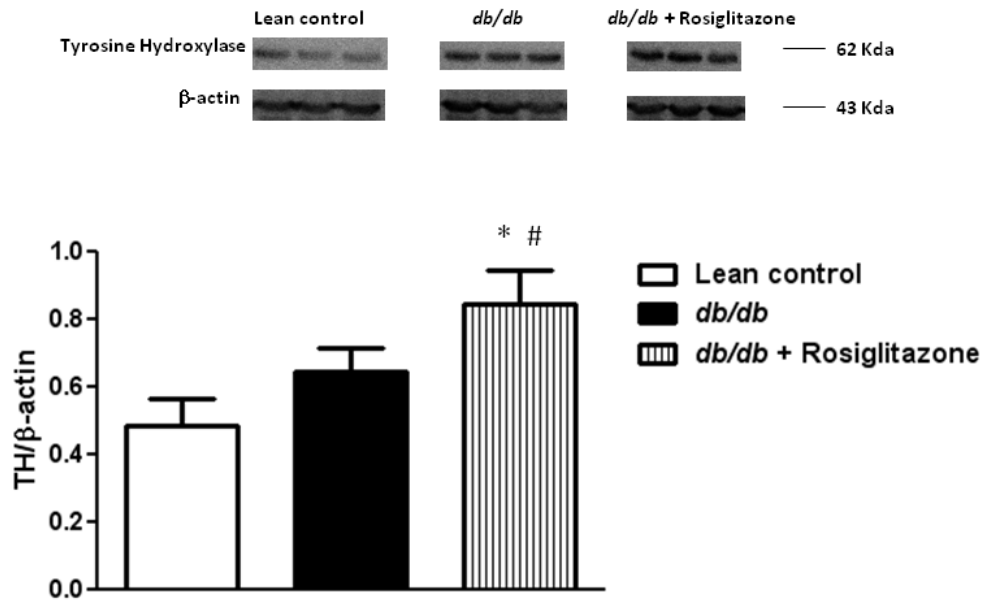


Fig. 26. Effect of rosiglitazone treatment on tyrosine hydroxylase (TH) expression in the hypothalamus of *db/db* mice. Unpaired t-test showed that there was no significant change in the TH expression in the *db/db* mice compared to age-matched lean control ($p > 0.05$). Each bar represents means \pm S.E.M. of data from $n = 3$ sample wells per group. * $p < 0.05$ vs. age-matched lean control mice; # $p < 0.05$ vs. age-matched *db/db* mice.

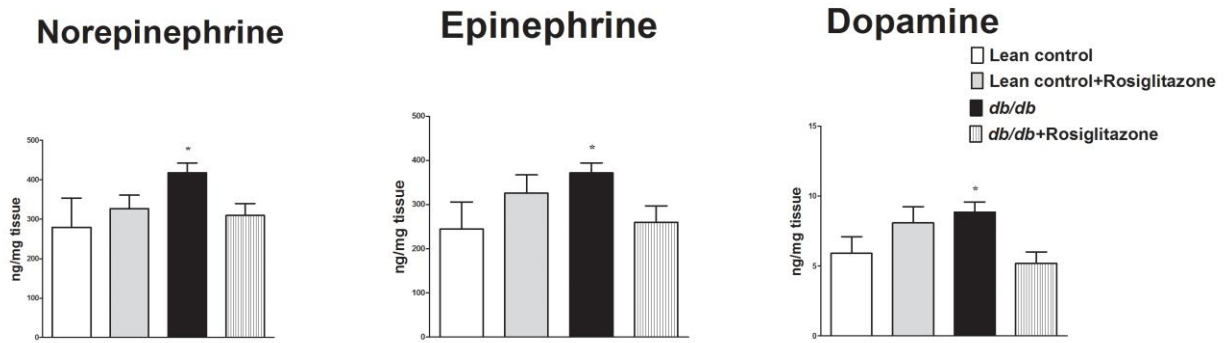


Fig. 27. Effect of rosiglitazone treatment on adrenal catecholamines levels of *db/db* mice. 1-way ANOVA showed that there was significant increase in the adrenal NE, E and DA levels in the *db/db* mice compared to age-matched lean control ($p < 0.05$). Each bar represents means \pm S.E.M. of data from $n = 6-7$ mice per group. 5-week rosiglitazone treatment helped to bring such changes to normal.

Table 2. Summary of type-2 diabetes related changes in DA metabolism and biosynthesis pattern in selective brain regions of *db/db* mice compared to age-matched lean controls.

Brain region	DA	HVA	DOPAC	HVA/DA	DOPAC/DA	TH/ β -actin
Frontal cortex	No change	Increased	Increased	Increased	Increased	No change
Inference: Changes in DA metabolism in the frontal cortex of <i>db/db</i> mice may be related to increased enzymatic activity of DA metabolizing enzymes and/or altered neuronal firing.						
Brain region	DA	HVA	DOPAC	HVA/DA	DOPAC/DA	TH/ β -actin
Amygdala	Increased	Increased	Increased	Decreased	Decreased	No change
Inference: Changes in DA metabolism in the amygdala of <i>db/db</i> mice may be related to increased enzymatic activity of DA metabolizing enzymes and/or altered neuronal firing.						

11. References

- Abizaid, A., Gao, Q., Horvath, T.L., 2006. Thoughts for food: brain mechanisms and peripheral energy balance. *Neuron* 51, 691-702.
- Ahima, R.S., Bjorbaek, C., Osei, S., Flier, J.S., 1999. Regulation of neuronal and glial proteins by leptin: implications for brain development. *Endocrinology* 140, 2755-2762.
- Ahima, R.S., Prabakaran, D., Mantzoros, C., Qu, D., Lowell, B., Maratos-Flier, E., Flier, J.S., 1996. Role of leptin in the neuroendocrine response to fasting. *Nature* 382, 250-252.
- Akil, M., Edgar, C.L., Pierri, J.N., Casali, S., Lewis, D.A., 2000. Decreased density of tyrosine hydroxylase-immunoreactive axons in the entorhinal cortex of schizophrenic subjects. *Biol. Psychiatry* 47, 361-370.
- Anderson, R.J., Freedland, K.E., Clouse, R.E., Lustman, P.J., 2001. The prevalence of comorbid depression in adults with diabetes: a meta-analysis. *Diabetes Care* 24, 1069-1078.
- Asakawa, A., Inui, A., Inui, T., Katsuura, G., Fujino, M.A., Kasuga, M., 2003. Leptin treatment ameliorates anxiety in ob/ob obese mice. *J. Diabetes Complications* 17, 105-107.
- Asakawa, A., Toyoshima, M., Inoue, K., Koizumi, A., 2007. Ins2Akita mice exhibit hyperphagia and anxiety behavior via the melanocortin system. *Int. J. Mol. Med.* 19, 649-652.
- Bacopoulos, N.C., Spokes, E.G., Bird, E.D., Roth, R.H., 1979. Antipsychotic drug action in schizophrenic patients: effect on cortical dopamine metabolism after long-term treatment. *Science* 205, 1405-1407.
- Baptista, T., Beaulieu, S., 2001. Leptin and antipsychotic drugs. *Br. J. Psychiatry* 179, 560-561.
- Barber, M., Kasturi, B.S., Austin, M.E., Patel, K.P., MohanKumar, S.M., MohanKumar, P.S., 2003. Diabetes-induced neuroendocrine changes in rats: role of brain monoamines, insulin and leptin. *Brain Res.* 964, 128-135.

- Bhatia, S.C., Saha, S., Manchanda, S.K., Nayar, U., 1997. Role of midbrain ventro-lateral tegmental area (VTA) adrenergic mechanisms in facilitation of hypothalamically-induced predatory attack behaviour. *Indian J. Exp. Biol.* 35, 332-337.
- Bjorklund, A., Lindvall, O., Nobin, A., 1975. Evidence of an incerto-hypothalamic dopamine neurone system in the rat. *Brain Res.* 89, 29-42.
- Blows, W.T., 2000a. Neurotransmitters of the brain: serotonin, noradrenaline (norepinephrine), and dopamine. *J. Neurosci. Nurs.* 32, 234-238.
- Blows, W.T., 2000b. The neurobiology of antidepressants. *J. Neurosci. Nurs.* 32, 177-180.
- Bohlen und, H.O., Zacher, C., Gass, P., Unsicker, K., 2006. Age-related alterations in hippocampal spines and deficiencies in spatial memory in mice. *J. Neurosci. Res.* 83, 525-531.
- Borsini, F., Meli, A., 1988. Is the forced swimming test a suitable model for revealing antidepressant activity? *Psychopharmacology (Berl)* 94, 147-160.
- Bowden, C., Cheetham, S.C., Lowther, S., Katona, C.L., Crompton, M.R., Horton, R.W., 1997. Reduced dopamine turnover in the basal ganglia of depressed suicides. *Brain Res.* 769, 135-140.
- Brodie, B.B., Shore, P.A., 1957. A concept for a role of serotonin and norepinephrine as chemical mediators in the brain. *Ann. N. Y. Acad. Sci.* 66, 631-642.
- Bruhwyler, J., Chleide, E., Liegeois, J.F., Delarge, J., Mercier, M., 1991. Effects of specific dopaminergic agonists and antagonists in the open-field test. *Pharmacol. Biochem. Behav.* 39, 367-371.
- Butler, S.G., Meegan, M.J., 2008. Recent developments in the design of anti-depressive therapies: targeting the serotonin transporter. *Curr. Med. Chem.* 15, 1737-1761.
- Buyse, M., Bado, A., Dauge, V., 2001. Leptin decreases feeding and exploratory behaviour via interactions with CCK1 receptors in the rat. *Neuropharmacology* 40, 818-825.

- Calapai, G., Corica, F., Corsonello, A., Sautebin, L., Di Rosa, M., Campo, G.M., Buemi, M., Mauro, V.N., Caputi, A.P., 1999. Leptin increases serotonin turnover by inhibition of brain nitric oxide synthesis. *J. Clin. Invest* 104, 975-982.
- Carter, T.L., Pedrini, S., Ghiso, J., Ehrlich, M.E., Gandy, S., 2006. Brain neprilysin activity and susceptibility to transgene-induced Alzheimer amyloidosis. *Neurosci. Lett.* 392, 235-239.
- Castaneda, T.R., Nogueiras, R., Muller, T.D., Krishna, R., Grant, E., Jones, A., Ottaway, N., Ananthakrishnan, G., Pfluger, P.T., Chaudhary, N., Solomon, M.B., Woods, S.C., Herman, J.P., Tschop, M.H., 2011. Decreased glucose tolerance and plasma adiponectin:resistin ratio in a mouse model of post-traumatic stress disorder. *Diabetologia* 54, 900-909.
- Chaouloff, F., 2000. Serotonin, stress and corticoids. *J. Psychopharmacol.* 14, 139-151.
- Chen, C.C., Yang, J.C., 1991. Effects of short and long-lasting diabetes mellitus on mouse brain monoamines. *Brain Res.* 552, 175-179.
- Chen, H., Charlat, O., Tartaglia, L.A., Woolf, E.A., Weng, X., Ellis, S.J., Lakey, N.D., Culpepper, J., Moore, K.J., Breitbart, R.E., Duyk, G.M., Tepper, R.I., Morgenstern, J.P., 1996. Evidence that the diabetes gene encodes the leptin receptor: identification of a mutation in the leptin receptor gene in db/db mice. *Cell* 84, 491-495.
- Cincotta, A.H., Meier, A.H., 1996. Bromocriptine (Ergoset) reduces body weight and improves glucose tolerance in obese subjects. *Diabetes Care* 19, 667-670.
- Cincotta, A.H., Tozzo, E., Scislowski, P.W., 1997. Bromocriptine/SKF38393 treatment ameliorates obesity and associated metabolic dysfunctions in obese (ob/ob) mice. *Life Sci.* 61, 951-956.
- Clarke, H.F., Walker, S.C., Crofts, H.S., Dalley, J.W., Robbins, T.W., Roberts, A.C., 2005. Prefrontal serotonin depletion affects reversal learning but not attentional set shifting. *J. Neurosci.* 25, 532-538.

- Cohen, D., Stolk, R.P., Grobbee, D.E., Gispen-de Wied, C.C., 2006. Hyperglycemia and diabetes in patients with schizophrenia or schizoaffective disorders. *Diabetes Care* 29, 786-791.
- Cohen, J.W., Krauss, N.A., 2003. Spending and service use among people with the fifteen most costly medical conditions, 1997. *Health Aff. (Millwood)* 22, 129-138.
- Collin, M., Hakansson-Ovesjo, M.L., Misane, I., Ogren, S.O., Meister, B., 2000. Decreased 5-HT transporter mRNA in neurons of the dorsal raphe nucleus and behavioral depression in the obese leptin-deficient ob/ob mouse. *Brain Res. Mol. Brain Res.* 81, 51-61.
- Collins, M.M., Corcoran, P., Perry, I.J., 2009. Anxiety and depression symptoms in patients with diabetes. *Diabet. Med.* 26, 153-161.
- Contreras, F., Foullioux, C., Pacheco, B., Maroun, C., Bolivar, H., Lares, M., Leal, E., Cano, R., Bermudez, V., Velasco, M., 2008a. Effect of drugs interacting with the dopaminergic receptors on glucose levels and insulin release in healthy and type 2 diabetic subjects. *Am. J. Ther.* 15, 397-402.
- Contreras, F., Foullioux, C., Pacheco, B., Maroun, C., Bolivar, H., Lares, M., Leal, E., Cano, R., Bermudez, V., Velasco, M., 2008b. Effect of drugs interacting with the dopaminergic receptors on glucose levels and insulin release in healthy and type 2 diabetic subjects. *Am. J. Ther.* 15, 397-402.
- Cottrell, E.C., Mercer, J.G., Ozanne, S.E., 2010. Postnatal development of hypothalamic leptin receptors. *Vitam. Horm.* 82, 201-217.
- Courtney, S.M., Petit, L., Maisog, J.M., Ungerleider, L.G., Haxby, J.V., 1998. An area specialized for spatial working memory in human frontal cortex. *Science* 279, 1347-1351.
- Crawley, J.N., Belknap, J.K., Collins, A., Crabbe, J.C., Frankel, W., Henderson, N., Hitzemann, R.J., Maxson, S.C., Miner, L.L., Silva, A.J., Wehner, J.M., Wynshaw-Boris, A., Paylor, R., 1997. Behavioral phenotypes of inbred mouse strains: implications and recommendations for molecular studies. *Psychopharmacology (Berl)* 132, 107-124.

- Cryan, J.F., Lucki, I., 2000. Antidepressant-like behavioral effects mediated by 5-Hydroxytryptamine(2C) receptors. *J. Pharmacol. Exp. Ther.* 295, 1120-1126.
- Davis, K.L., Kahn, R.S., Ko, G., Davidson, M., 1991. Dopamine in schizophrenia: a review and reconceptualization. *Am. J. Psychiatry* 148, 1474-1486.
- Davis, M., 1993. Pharmacological analysis of fear-potentiated startle. *Braz. J. Med. Biol. Res.* 26, 235-260.
- De Hert, M.A., Cohen, D., 2009. Antipsychotic use and risk for hyperglycemia in elderly patients with DM. *Arch. Intern. Med.* 169, 2165-2166.
- de la Monte, S.M., Longato, L., Tong, M., Wands, J.R., 2009. Insulin resistance and neurodegeneration: roles of obesity, type 2 diabetes mellitus and non-alcoholic steatohepatitis. *Curr. Opin. Investig. Drugs* 10, 1049-1060.
- de Leeuw van Weenen JE, Parlevliet, E.T., Maechler, P., Havekes, L.M., Romijn, J.A., Ouwens, D.M., Pijl, H., Guigas, B., 2010. The dopamine receptor D2 agonist bromocriptine inhibits glucose-stimulated insulin secretion by direct activation of the α_2 -adrenergic receptors in beta cells. *Biochem. Pharmacol.* 79, 1827-1836.
- Dickerson, F., Brown, C.H., Fang, L., Goldberg, R.W., Kreyenbuhl, J., Wohlheiter, K., Dixon, L., 2008. Quality of life in individuals with serious mental illness and type 2 diabetes. *Psychosomatics* 49, 109-114.
- Dickinson, D., Gold, J.M., Dickerson, F.B., Medoff, D., Dixon, L.B., 2008. Evidence of exacerbated cognitive deficits in schizophrenia patients with comorbid diabetes. *Psychosomatics* 49, 123-131.
- Dunlop, B.W., Nemeroff, C.B., 2007. The role of dopamine in the pathophysiology of depression. *Arch. Gen. Psychiatry* 64, 327-337.
- Dwyer, D.S., Bradley, R.J., Kablinger, A.S., Freeman, A.M., III, 2001. Glucose metabolism in relation to schizophrenia and antipsychotic drug treatment. *Ann. Clin. Psychiatry* 13, 103-113.

- Dwyer, D.S., Donohoe, D., 2003. Induction of hyperglycemia in mice with atypical antipsychotic drugs that inhibit glucose uptake. *Pharmacol. Biochem. Behav.* 75, 255-260.
- Dziedzicka-Wasylewska, M., Rogoz, R., Klimek, V., Maj, J., 1997. Repeated administration of antidepressant drugs affects the levels of mRNA coding for D1 and D2 dopamine receptors in the rat brain. *J. Neural Transm.* 104, 515-524.
- Eaton, W.W., Armenian, H., Gallo, J., Pratt, L., Ford, D.E., 1996. Depression and risk for onset of type II diabetes. A prospective population-based study. *Diabetes Care* 19, 1097-1102.
- Eissa Ahmed, A.A., Al-Rasheed, N.M., Al-Rasheed, N.M., 2009. Antidepressant-like effects of rosiglitazone, a PPARgamma agonist, in the rat forced swim and mouse tail suspension tests. *Behav. Pharmacol.* 20, 635-642.
- El-Amouri, S.S., Zhu, H., Yu, J., Marr, R., Verma, I.M., Kindy, M.S., 2008. Neprilysin: an enzyme candidate to slow the progression of Alzheimer's disease. *Am. J. Pathol.* 172, 1342-1354.
- Ellenbroek, B.A., Lubbers, L.J., Cools, A.R., 2002. The role of hippocampal dopamine receptors in prepulse inhibition. *Eur. J. Neurosci.* 15, 1237-1243.
- Elmquist, J.K., Maratos-Flier, E., Saper, C.B., Flier, J.S., 1998. Unraveling the central nervous system pathways underlying responses to leptin. *Nat. Neurosci.* 1, 445-450.
- Engstrom, G., Alling, C., Blennow, K., Regnell, G., Traskman-Bendz, L., 1999. Reduced cerebrospinal HVA concentrations and HVA/5-HIAA ratios in suicide attempters. Monoamine metabolites in 120 suicide attempters and 47 controls. *Eur. Neuropsychopharmacol.* 9, 399-405.
- Engum, A., 2007. The role of depression and anxiety in onset of diabetes in a large population-based study. *J. Psychosom. Res.* 62, 31-38.
- Fadok, J.P., Dickerson, T.M., Palmiter, R.D., 2009. Dopamine is necessary for cue-dependent fear conditioning. *J. Neurosci.* 29, 11089-11097.

- Farooqui, S.M., Brock, J.W., Onaivi, E.S., Hamdi, A., Prasad, C., 1994. Differential modulation of dopaminergic systems in the rat brain by dietary protein. *Neurochem. Res.* 19, 167-176.
- Farr, S.A., Banks, W.A., Morley, J.E., 2006. Effects of leptin on memory processing. *Peptides* 27, 1420-1425.
- Fei, H., Okano, H.J., Li, C., Lee, G.H., Zhao, C., Darnell, R., Friedman, J.M., 1997. Anatomic localization of alternatively spliced leptin receptors (Ob-R) in mouse brain and other tissues. *Proc. Natl. Acad. Sci. U. S. A* 94, 7001-7005.
- Fetissov, S., Marsais, F., Nicolaidis, S., Calas, A., 1997. Expression of tyrosine hydroxylase in magnocellular hypothalamic neurons of obese (fa / fa) and lean heterozygous (Fa / fa) Zucker rats. *Brain Res. Mol. Brain Res.* 50, 314-318.
- Fetissov, S.O., Meguid, M.M., Sato, T., Zhang, L.H., 2002. Expression of dopaminergic receptors in the hypothalamus of lean and obese Zucker rats and food intake. *Am. J. Physiol Regul. Integr. Comp Physiol* 283, R905-R910.
- Figlewicz, D.P., Brot, M.D., McCall, A.L., Szot, P., 1996. Diabetes causes differential changes in CNS noradrenergic and dopaminergic neurons in the rat: a molecular study. *Brain Res.* 736, 54-60.
- Figlewicz, D.P., Evans, S.B., Murphy, J., Hoen, M., Baskin, D.G., 2003. Expression of receptors for insulin and leptin in the ventral tegmental area/substantia nigra (VTA/SN) of the rat. *Brain Res.* 964, 107-115.
- Finger, B.C., Dinan, T.G., Cryan, J.F., 2010. Leptin-deficient mice retain normal appetitive spatial learning yet exhibit marked increases in anxiety-related behaviours. *Psychopharmacology (Berl)* 210, 559-568.
- Finn, P.D., Cunningham, M.J., Rickard, D.G., Clifton, D.K., Steiner, R.A., 2001. Serotonergic neurons are targets for leptin in the monkey. *J. Clin. Endocrinol. Metab* 86, 422-426.

- Fisher, L., Skaff, M.M., Mullan, J.T., Arean, P., Glasgow, R., Masharani, U., 2008. A longitudinal study of affective and anxiety disorders, depressive affect and diabetes distress in adults with Type 2 diabetes. *Diabet. Med.* 25, 1096-1101.
- Friedman, J.M., Halaas, J.L., 1998. Leptin and the regulation of body weight in mammals. *Nature* 395, 763-770.
- Fulton, S., Pissios, P., Manchon, R.P., Stiles, L., Frank, L., Pothos, E.N., Maratos-Flier, E., Flier, J.S., 2006. Leptin regulation of the mesoaccumbens dopamine pathway. *Neuron* 51, 811-822.
- Gainetdinov, R.R., 2007. Mesolimbic dopamine in obesity and diabetes. *Am. J. Physiol Regul. Integr. Comp Physiol* 293, R601-R602.
- Garcia-Tornadu, I., Ornstein, A.M., Chamson-Reig, A., Wheeler, M.B., Hill, D.J., Arany, E., Rubinstein, M., Becu-Villalobos, D., 2010a. Disruption of the dopamine d2 receptor impairs insulin secretion and causes glucose intolerance. *Endocrinology* 151, 1441-1450.
- Garcia-Tornadu, I., Ornstein, A.M., Chamson-Reig, A., Wheeler, M.B., Hill, D.J., Arany, E., Rubinstein, M., Becu-Villalobos, D., 2010b. Disruption of the dopamine d2 receptor impairs insulin secretion and causes glucose intolerance. *Endocrinology* 151, 1441-1450.
- Garris, D.R., 1988. Reproductive tract and pancreatic norepinephrine levels in pre- and overt-diabetic C57BL/KsJ mice: relationship to body weight, blood glucose, serum insulin, and reproductive dysfunction. *Proc. Soc. Exp. Biol. Med.* 189, 79-83.
- Garris, D.R., 1990. Age- and diabetes-associated alterations in regional brain norepinephrine concentrations and adrenergic receptor populations in C57BL/KsJ mice. *Brain Res. Dev. Brain Res.* 51, 161-166.
- Garris, D.R., 1995. Developmental and regional changes in brain norepinephrine levels in diabetic C57BL/KsJ mice: effects of estradiol and progesterone. *Brain Res. Dev. Brain Res.* 89, 314-319.

- Gaziano, J.M., Cincotta, A.H., O'Connor, C.M., Ezrokhi, M., Rutty, D., Ma, Z.J., Scranton, R.E., 2010. Randomized clinical trial of quick-release bromocriptine among patients with type 2 diabetes on overall safety and cardiovascular outcomes. *Diabetes Care* 33, 1503-1508.
- Gispen, W.H., Biessels, G.J., 2000. Cognition and synaptic plasticity in diabetes mellitus. *Trends Neurosci.* 23, 542-549.
- Golbidi, S., Ebadi, S.A., Laher, I., 2011. Antioxidants in the treatment of diabetes. *Curr. Diabetes Rev.* 7, 106-125.
- Gold, P.W., Chrousos, G.P., 2002. Organization of the stress system and its dysregulation in melancholic and atypical depression: high vs low CRH/NE states. *Mol. Psychiatry* 7, 254-275.
- Guest, P.C., Schwarz, E., Krishnamurthy, D., Harris, L.W., Leweke, F.M., Rothermundt, M., van Beveren, N.J., Spain, M., Barnes, A., Steiner, J., Rahmoune, H., Bahn, S., 2011. Altered levels of circulating insulin and other neuroendocrine hormones associated with the onset of schizophrenia. *Psychoneuroendocrinology*.
- Guiard, B.P., El Mansari, M., Blier, P., 2009. Prospect of a Dopamine Contribution in the Next Generation of Antidepressant Drugs: The Triple Reuptake Inhibitors. *Curr. Drug Targets*.
- Gupta, G., Azam, M., Baquer, N.Z., 1992. Effect of experimental diabetes on the catecholamine metabolism in rat brain. *J. Neurochem.* 58, 95-100.
- Hafez, D., Huang, J.Y., Huynh, A.M., Valtierra, S., Rockenstein, E., Bruno, A.M., Lu, B., DesGroseillers, L., Masliah, E., Marr, R.A., 2011. Neprilysin-2 is an important beta-amyloid degrading enzyme. *Am. J. Pathol.* 178, 306-312.
- Hall, P.A., Rodin, G.M., Vallis, T.M., Perkins, B.A., 2009. The consequences of anxious temperament for disease detection, self-management behavior, and quality of life in Type 2 diabetes mellitus. *J. Psychosom. Res.* 67, 297-305.

- Harris, R.B., Zhou, J., Redmann, S.M., Jr., Smagin, G.N., Smith, S.R., Rodgers, E., Zachwieja, J.J., 1998. A leptin dose-response study in obese (ob/ob) and lean (+/?) mice. *Endocrinology* 139, 8-19.
- Hata, T., Nishikawa, H., Itoh, E., Funakami, Y., 2001. Anxiety-like behavior in elevated plus-maze tests in repeatedly cold-stressed mice. *Jpn. J. Pharmacol.* 85, 189-196.
- Hesse, D., Dunn, M., Heldmaier, G., Klingenspor, M., Rozman, J., 2009. Behavioural mechanisms affecting energy regulation in mice prone or resistant to diet- induced obesity. *Physiol Behav.*
- Hiles, B.W., 1956. Hyperglycemia and glucosuria following chlorpromazine therapy. In: p. 1651.
- Hirano, S., Miyata, S., Kamei, J., 2007. Antidepressant-like effect of leptin in streptozotocin-induced diabetic mice. *Pharmacol. Biochem. Behav.* 86, 27-31.
- Hoebel, B.G., Hernandez, L., Schwartz, D.H., Mark, G.P., Hunter, G.A., 1989. Microdialysis studies of brain norepinephrine, serotonin, and dopamine release during ingestive behavior. Theoretical and clinical implications. *Ann. N. Y. Acad. Sci.* 575, 171-191.
- Hogan, P., Dall, T., Nikolov, P., 2003. Economic costs of diabetes in the US in 2002. *Diabetes Care* 26, 917-932.
- Holguin, F., Rojas, M., Hart, C.M., 2007. The peroxisome proliferator activated receptor gamma (PPARgamma) ligand rosiglitazone modulates bronchoalveolar lavage levels of leptin, adiponectin, and inflammatory cytokines in lean and obese mice. *Lung* 185, 367-372.
- Holt, R.I., 2004. Diagnosis, epidemiology and pathogenesis of diabetes mellitus: an update for psychiatrists. *Br. J. Psychiatry Suppl* 47, S55-S63.
- Howes, O.D., Kapur, S., 2009. The dopamine hypothesis of schizophrenia: version III--the final common pathway. *Schizophr. Bull.* 35, 549-562.
- Huang, T.J., Price, S.A., Chilton, L., Calcutt, N.A., Tomlinson, D.R., Verkhatsky, A., Fernyhough, P., 2003. Insulin prevents depolarization of the mitochondrial inner membrane in sensory neurons of type 1 diabetic rats in the presence of sustained hyperglycemia. *Diabetes* 52, 2129-2136.

- Iwata, N., Tsubuki, S., Takaki, Y., Shirotani, K., Lu, B., Gerard, N.P., Gerard, C., Hama, E., Lee, H.J., Saido, T.C., 2001. Metabolic regulation of brain Abeta by neprilysin. *Science* 292, 1550-1552.
- Jacob, R.J., Fan, X., Evans, M.L., Dziura, J., Sherwin, R.S., 2002. Brain glucose levels are elevated in chronically hyperglycemic diabetic rats: no evidence for protective adaptation by the blood brain barrier. *Metabolism* 51, 1522-1524.
- Johnson, G.D., Stevenson, T., Ahn, K., 1999. Hydrolysis of peptide hormones by endothelin-converting enzyme-1. A comparison with neprilysin. *J. Biol. Chem.* 274, 4053-4058.
- Katon, W.J., 2008. The comorbidity of diabetes mellitus and depression. *Am. J. Med.* 121, S8-15.
- Katon, W.J., Lin, E.H., Russo, J., Von, K.M., Ciechanowski, P., Simon, G., Ludman, E., Bush, T., Young, B., 2004. Cardiac risk factors in patients with diabetes mellitus and major depression. *J. Gen. Intern. Med.* 19, 1192-1199.
- Katz, R.J., 1982. Animal model of depression: pharmacological sensitivity of a hedonic deficit. *Pharmacol. Biochem. Behav.* 16, 965-968.
- Kawakami, N., Takatsuka, N., Shimizu, H., Ishibashi, H., 1999. Depressive symptoms and occurrence of type 2 diabetes among Japanese men. *Diabetes Care* 22, 1071-1076.
- King, T.S., Rohrbach, D.H., 1990. Reduced aminergic synthesis in the hypothalamus of the infertile, genetically diabetic (C57BL/KsJ-db/db) male mouse. *Exp. Brain Res.* 81, 619-625.
- Klempin, F., Babu, H., Tonelli, D.P., Alarcon, E., Fabel, K., Kempermann, G., 2010. Oppositional Effects of Serotonin Receptors 5-HT_{1a}, 2, and 2c in the Regulation of Adult Hippocampal Neurogenesis. *Front Mol. Neurosci.* 3.
- Knol, M.J., Twisk, J.W., Beekman, A.T., Heine, R.J., Snoek, F.J., Pouwer, F., 2006. Depression as a risk factor for the onset of type 2 diabetes mellitus. A meta-analysis. *Diabetologia* 49, 837-845.

- Kok, P., Roelfsema, F., Frolich, M., van Pelt, J., Meinders, A.E., Pijl, H., 2006. Activation of dopamine D2 receptors lowers circadian leptin concentrations in obese women. *J. Clin. Endocrinol. Metab* 91, 3236-3240.
- Koopmans, B., Pouwer, F., de Bie, R.A., van Rooij, E.S., Leusink, G.L., Pop, V.J., 2009. Depressive symptoms are associated with physical inactivity in patients with type 2 diabetes. The DIAZOB Primary Care Diabetes study. *Fam. Pract.* 26, 171-173.
- Labad, J., Price, J.F., Strachan, M.W., Fowkes, F.G., Ding, J., Deary, I.J., Lee, A.J., Frier, B.M., Seckl, J.R., Walker, B.R., Reynolds, R.M., 2010. Symptoms of depression but not anxiety are associated with central obesity and cardiovascular disease in people with type 2 diabetes: the Edinburgh Type 2 Diabetes Study. *Diabetologia* 53, 467-471.
- Lackovic, Z., Salkovic, M., Kuci, Z., Relja, M., 1990. Effect of long-lasting diabetes mellitus on rat and human brain monoamines. *J. Neurochem.* 54, 143-147.
- Lambert, B.L., Cunningham, F.E., Miller, D.R., Dalack, G.W., Hur, K., 2006. Diabetes risk associated with use of olanzapine, quetiapine, and risperidone in veterans health administration patients with schizophrenia. *Am. J. Epidemiol.* 164, 672-681.
- Laposky, A.D., Bradley, M.A., Williams, D.L., Bass, J., Turek, F.W., 2008. Sleep-wake regulation is altered in leptin-resistant (db/db) genetically obese and diabetic mice. *Am. J. Physiol Regul. Integr. Comp Physiol* 295, R2059-R2066.
- Lazar, N.L., Rajakumar, N., Cain, D.P., 2008. Injections of NGF into neonatal frontal cortex decrease social interaction as adults: A rat model of schizophrenia. *Schizophrenia Bulletin* 34, 127-136.
- Lemierre, S., Rouch, C., Nicolaidis, S., Orosco, M., 1998. Combined effect of obesity and aging on feeding-induced monoamine release in the rostromedial hypothalamus of the Zucker rat. *Int. J. Obes. Relat Metab Disord.* 22, 993-999.

- Levin, N., Nelson, C., Gurney, A., Vandlen, R., de Sauvage, F., 1996. Decreased food intake does not completely account for adiposity reduction after ob protein infusion. *Proc. Natl. Acad. Sci. U. S. A* 93, 1726-1730.
- Levitt Katz, L.E., Swami, S., Abraham, M., Murphy, K.M., Jawad, A.F., Knight-Menci, H., Berkowitz, R., 2005. Neuropsychiatric disorders at the presentation of type 2 diabetes mellitus in children. *Pediatr. Diabetes* 6, 84-89.
- Li, X.L., Aou, S., Oomura, Y., Hori, N., Fukunaga, K., Hori, T., 2002. Impairment of long-term potentiation and spatial memory in leptin receptor-deficient rodents. *Neuroscience* 113, 607-615.
- Liang, Y., Lubkin, M., Sheng, H., Scislawski, P.W., Cincotta, A.H., 1998. Dopamine agonist treatment ameliorates hyperglycemia, hyperlipidemia, and the elevated basal insulin release from islets of ob/ob mice. *Biochim. Biophys. Acta* 1405, 1-13.
- Lin, C.Y., Higginbotham, D.A., Judd, R.L., White, B.D., 2002. Central leptin increases insulin sensitivity in streptozotocin-induced diabetic rats. *Am. J. Physiol Endocrinol. Metab* 282, E1084-E1091.
- Lin, E.H., Katon, W., Von Korff, M., Rutter, C., Simon, G.E., Oliver, M., Ciechanowski, P., Ludman, E.J., Bush, T., Young, B., 2004. Relationship of depression and diabetes self-care, medication adherence, and preventive care. *Diabetes Care* 27, 2154-2160.
- Lin, P.I., Shuldiner, A.R., 2010. Rethinking the genetic basis for comorbidity of schizophrenia and type 2 diabetes. *Schizophr. Res.* 123, 234-243.
- Lipscombe, L.L., Levesque, L., Gruneir, A., Fischer, H.D., Juurlink, D.N., Gill, S.S., Herrmann, N., Hux, J.E., Anderson, G.M., Rochon, P.A., 2009. Antipsychotic drugs and hyperglycemia in older patients with diabetes. *Arch. Intern. Med.* 169, 1282-1289.
- Liu, Y., Liu, L., Lu, S., Wang, D., Liu, X., Xie, L., Wang, G., 2011. Impaired amyloid beta-degrading enzymes in brain of streptozotocin-induced diabetic rats. *J. Endocrinol. Invest* 34, 26-31.

- Lu, M., Sarruf, D.A., Talukdar, S., Sharma, S., Li, P., Bandyopadhyay, G., Nalbandian, S., Fan, W., Gayen, J.R., Mahata, S.K., Webster, N.J., Schwartz, M.W., Olefsky, J.M., 2011. Brain PPAR-gamma promotes obesity and is required for the insulin-sensitizing effect of thiazolidinediones. *Nat. Med.* 17, 618-622.
- Lu, X.Y., Kim, C.S., Frazer, A., Zhang, W., 2006. Leptin: a potential novel antidepressant. *Proc. Natl. Acad. Sci. U. S. A* 103, 1593-1598.
- Lucki, I., Dalvi, A., Mayorga, A.J., 2001. Sensitivity to the effects of pharmacologically selective antidepressants in different strains of mice. *Psychopharmacology (Berl)* 155, 315-322.
- Luo, S., Luo, J., Meier, A.H., Cincotta, A.H., 1997. Dopaminergic neurotoxin administration to the area of the suprachiasmatic nuclei induces insulin resistance. *Neuroreport* 8, 3495-3499.
- Lustman, P.J., Anderson, R.J., Freedland, K.E., de, G.M., Carney, R.M., Clouse, R.E., 2000. Depression and poor glycemic control: a meta-analytic review of the literature. *Diabetes Care* 23, 934-942.
- Ma, M.X., Chen, Y.M., He, J., Zeng, T., Wang, J.H., 2007. Effects of morphine and its withdrawal on Y-maze spatial recognition memory in mice. *Neuroscience* 147, 1059-1065.
- Macedo, D.S., Vasconcelos, S.M., Belchior, L.D., Santos, R.S., Viana, G.S., Sousa, F.C., 2004. Alterations in monoamine levels after cocaine-induced status epilepticus and death in striatum and prefrontal cortex of mice. *Neurosci. Lett.* 362, 185-188.
- Mach, M., Grubbs, R.D., Price, W.A., Nagaoka, M., Dubovicky, M., Lucot, J.B., 2008. Delayed behavioral and endocrine effects of sarin and stress exposure in mice. *J. Appl. Toxicol.* 28, 132-139.
- Maeda, T., Kiguchi, N., Fukazawa, Y., Yamamoto, A., Ozaki, M., Kishioka, S., 2007. Peroxisome proliferator-activated receptor gamma activation relieves expression of behavioral sensitization to methamphetamine in mice. *Neuropsychopharmacology* 32, 1133-1140.

- Malone, J.I., Hanna, S., Saporta, S., Mervis, R.F., Park, C.R., Chong, L., Diamond, D.M., 2008. Hyperglycemia not hypoglycemia alters neuronal dendrites and impairs spatial memory. *Pediatr. Diabetes* 9, 531-539.
- Maren, S., 2001a. Is there savings for pavlovian fear conditioning after neurotoxic basolateral amygdala lesions in rats? *Neurobiol. Learn. Mem.* 76, 268-283.
- Maren, S., 2001b. Neurobiology of Pavlovian fear conditioning. *Annu. Rev. Neurosci.* 24, 897-931.
- Marr, R.A., Guan, H., Rockenstein, E., Kindy, M., Gage, F.H., Verma, I., Masliah, E., Hersh, L.B., 2004. Neprilysin regulates amyloid Beta peptide levels. *J. Mol. Neurosci.* 22, 5-11.
- Matochik, J.A., London, E.D., Yildiz, B.O., Ozata, M., Caglayan, S., DePaoli, A.M., Wong, M.L., Licinio, J., 2005. Effect of leptin replacement on brain structure in genetically leptin-deficient adults. *J. Clin. Endocrinol. Metab* 90, 2851-2854.
- Maudsley H, 1879. The pathology of mind. In. Macmillan, London.
- Mayorga, A.J., Lucki, I., 2001. Limitations on the use of the C57BL/6 mouse in the tail suspension test. *Psychopharmacology (Berl)* 155, 110-112.
- Meguid, M.M., Fetissov, S.O., Blaha, V., Yang, Z.J., 2000. Dopamine and serotonin VMN release is related to feeding status in obese and lean Zucker rats. *Neuroreport* 11, 2069-2072.
- Mehler-Wex, C., Riederer, P., Gerlach, M., 2006. Dopaminergic dysbalance in distinct basal ganglia neurocircuits: Implications for the pathophysiology of Parkinson's disease, schizophrenia and attention deficit hyperactivity disorder. *Neurotoxicity Research* 10, 167-179.
- Mercer, J.G., Hoggard, N., Williams, L.M., Lawrence, C.B., Hannah, L.T., Trayhurn, P., 1996. Localization of leptin receptor mRNA and the long form splice variant (Ob-Rb) in mouse hypothalamus and adjacent brain regions by in situ hybridization. *FEBS Lett.* 387, 113-116.

- Miller, S.A., Mancuso, C.A., Boutin-Foster, C., Michelen, W., Lean-Long, C., Foote, B., Charlson, M.E., 2011. Associations between posttraumatic stress disorder and hemoglobin A1(C) in low-income minority patients with diabetes. *Gen. Hosp. Psychiatry* 33, 116-122.
- Moncrieff, J., 2009. A critique of the dopamine hypothesis of schizophrenia and psychosis. *Harv. Rev. Psychiatry* 17, 214-225.
- Moriya, M., Okumura, T., Takahashi, N., Yamagata, K., Motomura, W., Kohgo, Y., 1999. An inverse correlation between serum leptin levels and hemoglobin A1c in patients with non-insulin dependent diabetes mellitus. *Diabetes Res. Clin. Pract.* 43, 187-191.
- Mukherjee, S., Decina, P., Bocola, V., Saraceni, F., Scapicchio, P., 1996. Diabetes mellitus in schizophrenic patients. *Comprehensive Psychiatry* 37, 68-73.
- Mukherjee, S., Schnur, D.B., Reddy, R., 1989. Family History of Type-2 Diabetes in Schizophrenic-Patients. *Lancet* 1, 495.
- Musselman, D.L., Betan, E., Larsen, H., Phillips, L.S., 2003. Relationship of depression to diabetes types 1 and 2: epidemiology, biology, and treatment. *Biol. Psychiatry* 54, 317-329.
- Nakasato, A., Nakatani, Y., Seki, Y., Tsujino, N., Umino, M., Arita, H., 2008. Swim stress exaggerates the hyperactive mesocortical dopamine system in a rodent model of autism. *Brain Research* 1193, 128-135.
- O'Malley, D., MacDonald, N., Mizielinska, S., Connolly, C.N., Irving, A.J., Harvey, J., 2007. Leptin promotes rapid dynamic changes in hippocampal dendritic morphology. *Mol. Cell Neurosci.* 35, 559-572.
- Ohta, R., Shigemura, N., Sasamoto, K., Koyano, K., Ninomiya, Y., 2003. Conditioned taste aversion learning in leptin-receptor-deficient db/db mice. *Neurobiol. Learn. Mem.* 80, 105-112.

- Orosco, M., Gerozissis, K., Rouch, C., Meile, M.J., Nicolaidis, S., 1995. Hypothalamic monoamines and insulin in relation to feeding in the genetically obese Zucker rat as revealed by microdialysis. *Obes. Res.* 3 Suppl 5, 655S-665S.
- Papapetropoulos, S., Ellul, J., Argyriou, A.A., Chroni, E., Lekka, N.P., 2006. The effect of depression on motor function and disease severity of Parkinson's disease. *Clin. Neurol. Neurosurg.* 108, 465-469.
- Paulmann, N., Grohmann, M., Voigt, J.P., Bert, B., Vowinckel, J., Bader, M., Skelin, M., Jevsek, M., Fink, H., Rupnik, M., Walther, D.J., 2009. Intracellular serotonin modulates insulin secretion from pancreatic beta-cells by protein serotonylation. *PLoS. Biol.* 7, e1000229.
- Paz-Filho, G.J., Babikian, T., Asarnow, R., Delibasi, T., Esposito, K., Erol, H.K., Wong, M.L., Licinio, J., 2008. Leptin replacement improves cognitive development. *PLoS. One.* 3, e3098.
- Pellemounter, M.A., Cullen, M.J., Baker, M.B., Hecht, R., Winters, D., Boone, T., Collins, F., 1995. Effects of the obese gene product on body weight regulation in ob/ob mice. *Science* 269, 540-543.
- Pellow, S., Chopin, P., File, S.E., Briley, M., 1985. Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *J. Neurosci. Methods* 14, 149-167.
- Peveler, R.C., Fairburn, C.G., 1989. Anorexia nervosa in association with diabetes mellitus--a cognitive-behavioural approach to treatment. *Behav. Res. Ther.* 27, 95-99.
- Pijl, H., 2003. Reduced dopaminergic tone in hypothalamic neural circuits: expression of a "thrifty" genotype underlying the metabolic syndrome? *Eur. J. Pharmacol.* 480, 125-131.
- Poirier, R., Wolfer, D.P., Welzl, H., Tracy, J., Galsworthy, M.J., Nitsch, R.M., Mohajeri, M.H., 2006. Neuronal neprilysin overexpression is associated with attenuation of Abeta-related spatial memory deficit. *Neurobiol. Dis.* 24, 475-483.

- Porsolt, R.D., Brossard, G., Hautbois, C., Roux, S., 2001. Rodent models of depression: forced swimming and tail suspension behavioral despair tests in rats and mice. *Curr. Protoc. Neurosci.* Chapter 8, Unit.
- Pouwer, F., 2009. Should we screen for emotional distress in type 2 diabetes mellitus? *Nat. Rev. Endocrinol.* 5, 665-671.
- Ramaswamy, K., Masand, P.S., Nasrallah, H.A., 2006. Do certain atypical antipsychotics increase the risk of diabetes? A critical review of 17 pharmacoepidemiologic studies. *Ann. Clin. Psychiatry* 18, 183-194.
- Rasgon, N.L., Kenna, H.A., Williams, K.E., Powers, B., Wroolie, T., Schatzberg, A.F., 2010. Rosiglitazone add-on in treatment of depressed patients with insulin resistance: a pilot study. *ScientificWorldJournal.* 10, 321-328.
- Remington, G., 2008. Alterations of dopamine and serotonin transmission in schizophrenia. *Prog. Brain Res.* 172, 117-140.
- Renard, C.E., Fiocco, A.J., Clenet, F., Hascoet, M., Bourin, M., 2001. Is dopamine implicated in the antidepressant-like effects of selective serotonin reuptake inhibitors in the mouse forced swimming test? *Psychopharmacology (Berl)* 159, 42-50.
- Robertson, S.D., Matthies, H.J., Owens, W.A., Sathananthan, V., Christianson, N.S., Kennedy, J.P., Lindsley, C.W., Daws, L.C., Galli, A., 2010. Insulin reveals Akt signaling as a novel regulator of norepinephrine transporter trafficking and norepinephrine homeostasis. *J. Neurosci.* 30, 11305-11316.
- Rosenzweig-Lipson, S., Dunlop, J., Marquis, K.L., 2007. 5-HT_{2C} receptor agonists as an innovative approach for psychiatric disorders. *Drug News Perspect.* 20, 565-571.

- Rutledge, L.P., Ngong, J.M., Kuperberg, J.M., Samaan, S.S., Soliman, K.F., Kolta, M.G., 2002. Dopaminergic system modulation of nociceptive response in long-term diabetic rats. *Pharmacol. Biochem. Behav.* 74, 1-9.
- Ryan, C.M., Freed, M.I., Rood, J.A., Cobitz, A.R., Waterhouse, B.R., Strachan, M.W., 2006. Improving metabolic control leads to better working memory in adults with type 2 diabetes. *Diabetes Care* 29, 345-351.
- Sadaghiani, M.S., Javadi-Paydar, M., Gharedaghi, M.H., Fard, Y.Y., Dehpour, A.R., 2011. Antidepressant-like effect of pioglitazone in the forced swimming test in mice: The role of PPAR-gamma receptor and nitric oxide pathway. *Behav. Brain Res.*
- Schwarz, E., Bahn, S., 2008. Cerebrospinal fluid: identification of diagnostic markers for schizophrenia. *Expert. Rev. Mol. Diagn.* 8, 209-216.
- Scislawski, P.W., Tozzo, E., Zhang, Y., Phaneuf, S., Prevelige, R., Cincotta, A.H., 1999. Biochemical mechanisms responsible for the attenuation of diabetic and obese conditions in ob/ob mice treated with dopaminergic agonists. *Int. J. Obes. Relat Metab Disord.* 23, 425-431.
- Scranton, R., Cincotta, A., 2010. Bromocriptine--unique formulation of a dopamine agonist for the treatment of type 2 diabetes. *Expert. Opin. Pharmacother.* 11, 269-279.
- Senador, D., Kanakamedala, K., Irigoyen, M.C., Morris, M., Elased, K.M., 2009. Cardiovascular and autonomic phenotype of db/db diabetic mice. *Exp. Physiol* 94, 648-658.
- Sentissi, O., Epelbaum, J., Olie, J.P., Poirier, M.F., 2008. Leptin and ghrelin levels in patients with schizophrenia during different antipsychotics treatment: a review. *Schizophr. Bull.* 34, 1189-1199.
- Sharma, A.N., Elased, K.M., Garrett, T.L., Lucot, J.B., 2010a. Neurobehavioral deficits in db/db diabetic mice. *Physiol Behav.* 101, 381-388.
- Sharma, A.N., Lucot, J.B., Garrett, T.L., Elased, K.M., 2010b. Depression and Psychosis-Like Behavior in db/db Diabetic Mice. *Diabetes* 59, A671-A672.

- Shor-Posner, G., Grinker, J.A., Marinescu, C., Leibowitz, S.F., 1985. Role of hypothalamic norepinephrine in control of meal patterns. *Physiol Behav.* 35, 209-214.
- Simson, P.E., Weiss, J.M., 1988a. Altered activity of the locus coeruleus in an animal model of depression. *Neuropsychopharmacology* 1, 287-295.
- Simson, P.E., Weiss, J.M., 1988b. Responsiveness of locus ceruleus neurons to excitatory stimulation is uniquely regulated by alpha 2 receptors. *Psychopharmacol. Bull.* 24, 349-354.
- Siuciak, J.A., Chapin, D.S., McCarthy, S.A., Guanowsky, V., Brown, J., Chiang, P., Marala, R., Patterson, T., Seymour, P.A., Swick, A., Iredale, P.A., 2007. CP-809,101, a selective 5-HT_{2C} agonist, shows activity in animal models of antipsychotic activity. *Neuropharmacology* 52, 279-290.
- Skidgel, R.A., Erdos, E.G., 2004. Angiotensin converting enzyme (ACE) and neprilysin hydrolyze neuropeptides: a brief history, the beginning and follow-ups to early studies. *Peptides* 25, 521-525.
- Stevenson, C.W., Gratton, A., 2004. Role of basolateral amygdala dopamine in modulating prepulse inhibition and latent inhibition in the rat. *Psychopharmacology (Berl)* 176, 139-145.
- Stranahan, A.M., Arumugam, T.V., Cutler, R.G., Lee, K., Egan, J.M., Mattson, M.P., 2008. Diabetes impairs hippocampal function through glucocorticoid-mediated effects on new and mature neurons. *Nat. Neurosci.* 11, 309-317.
- Stranahan, A.M., Lee, K., Martin, B., Maudsley, S., Golden, E., Cutler, R.G., Mattson, M.P., 2009. Voluntary exercise and caloric restriction enhance hippocampal dendritic spine density and BDNF levels in diabetic mice. *Hippocampus* 19, 951-961.
- Strekalova, T., Spanagel, R., Bartsch, D., Henn, F.A., Gass, P., 2004. Stress-induced anhedonia in mice is associated with deficits in forced swimming and exploration. *Neuropsychopharmacology* 29, 2007-2017.
- Sun, Y., Asnicar, M., Smith, R.G., 2007. Central and peripheral roles of ghrelin on glucose homeostasis. *Neuroendocrinology* 86, 215-228.

- Suomalainen, M., Mannisto, P.T., 1998. Lack of effect of leptin on the behaviour of mice predicting the level of anxiety and depression. *Pharmacol. Toxicol.* 83, 139-142.
- Surwit, R.S., Feinglos, M.N., 1988. Stress and autonomic nervous system in type II diabetes. A hypothesis. *Diabetes Care* 11, 83-85.
- Surwit, R.S., Schneider, M.S., 1993. Role of stress in the etiology and treatment of diabetes mellitus. *Psychosom. Med.* 55, 380-393.
- Surwit, R.S., van Tilburg, M.A., Zucker, N., McCaskill, C.C., Parekh, P., Feinglos, M.N., Edwards, C.L., Williams, P., Lane, J.D., 2002. Stress management improves long-term glycemic control in type 2 diabetes. *Diabetes Care* 25, 30-34.
- Taicher, G.Z., Tinsley, F.C., Reidman, A., Heiman, M.L., 2003. Quantitative magnetic resonance (QMR) method for bone and whole-body-composition analysis. *Anal. Bioanal. Chem.* 377, 990-1002.
- Thal, S.C., Heinemann, M., Luh, C., Pieter, D., Werner, C., Engelhard, K., 2011. Pioglitazone Reduces Secondary Brain Damage after Experimental Brain Trauma by PPAR-gamma-Independent Mechanisms. *J. Neurotrauma* 28, 983-993.
- Trayhurn, P., 1979. Thermoregulation in the diabetic-obese (db/db) mouse. The role of non-shivering thermogenesis in energy balance. *Pflugers Arch.* 380, 227-232.
- Trento, M., Kucich, C., Tibaldi, P., Gennari, S., Tedesco, S., Balbo, M., Arvat, E., Cavallo, F., Ghigo, E., Porta, M., 2010. A study of central serotonergic activity in healthy subjects and patients with Type 2 diabetes treated by traditional one-to-one care or Group Care. *J. Endocrinol. Invest* 33, 624-628.
- Tsai, M.H., Wei, I.H., Jiang-Shieh, Y.F., Jou, M.J., Ko, M.H., Chen, H.M., Wu, C.H., 2008. Expression of protein gene product 9.5, tyrosine hydroxylase and serotonin in the pineal gland of rats with streptozotocin-induced diabetes. *Neurosci. Res.* 60, 233-243.

- Tsuruta, R., Fujita, M., Ono, T., Koda, Y., Koga, Y., Yamamoto, T., Nanba, M., Shitara, M., Kasaoka, S., Maruyama, I., Yuasa, M., Maekawa, T., 2010. Hyperglycemia enhances excessive superoxide anion radical generation, oxidative stress, early inflammation, and endothelial injury in forebrain ischemia/reperfusion rats. *Brain Res.* 1309, 155-163.
- Umrani, D.N., Banday, A.A., Hussain, T., Lokhandwala, M.F., 2002. Rosiglitazone treatment restores renal dopamine receptor function in obese Zucker rats. *Hypertension* 40, 880-885.
- van Nimwegen, L.J., Storosum, J.G., Blumer, R.M., Allick, G., Venema, H.W., de Haan, L., Becker, H., van Amelsvoort, T., Ackermans, M.T., Fliers, E., Serlie, M.J., Sauerwein, H.P., 2008. Hepatic insulin resistance in antipsychotic naive schizophrenic patients: stable isotope studies of glucose metabolism. *J. Clin. Endocrinol. Metab* 93, 572-577.
- van Winkel, R., De Hert, M., Wampers, M., Van Eyck, D., Hanssens, L., Scheen, A., Peuskens, J., 2008. Major changes in glucose metabolism, including new-onset diabetes, within 3 months after initiation of or switch to atypical antipsychotic medication in patients with schizophrenia and schizoaffective disorder. *J. Clin. Psychiatry* 69, 472-479.
- Venkatasubramanian, G., Arasappa, R., Christopher, R., Gangadhar, B.N., 2010. Neuropharmacology of schizophrenia: is there a role for leptin? *Clin. Chem. Lab Med.* 48, 895-896.
- Ventura, R., Cabib, S., Alcaro, A., Orsini, C., Puglisi-Allegra, S., 2003. Norepinephrine in the prefrontal cortex is critical for amphetamine-induced reward and mesoaccumbens dopamine release. *J. Neurosci.* 23, 1879-1885.
- Villablanca, J.R., 2010. Why do we have a caudate nucleus? *Acta Neurobiol. Exp. (Wars.)* 70, 95-105.
- Volkow, N.D., Logan, J., Fowler, J.S., Wang, G.J., Gur, R.C., Wong, C., Felder, C., Gatley, S.J., Ding, Y.S., Hitzemann, R., Pappas, N., 2000. Association between age-related decline in brain dopamine activity and impairment in frontal and cingulate metabolism. *Am. J. Psychiatry* 157, 75-80.

- Wade, J.M., Juneja, P., MacKay, A.W., Graham, J., Havel, P.J., Tecott, L.H., Goulding, E.H., 2008. Synergistic impairment of glucose homeostasis in ob/ob mice lacking functional serotonin 2C receptors. *Endocrinology* 149, 955-961.
- Walther, T., Albrecht, D., Becker, M., Schubert, M., Kouznetsova, E., Wiesner, B., Maul, B., Schliebs, R., Grecksch, G., Furkert, J., Sterner-Kock, A., Schultheiss, H.P., Becker, A., Siems, W.E., 2009. Improved learning and memory in aged mice deficient in amyloid beta-degrading neutral endopeptidase. *PLoS. One.* 4, e4590.
- Wang, G.J., Volkow, N.D., Logan, J., Pappas, N.R., Wong, C.T., Zhu, W., Netusil, N., Fowler, J.S., 2001. Brain dopamine and obesity. *Lancet* 357, 354-357.
- Weinberger, D.R., Berman, K.F., Illowsky, B.P., 1988. Physiological dysfunction of dorsolateral prefrontal cortex in schizophrenia. III. A new cohort and evidence for a monoaminergic mechanism. *Arch. Gen. Psychiatry* 45, 609-615.
- Weiss, T., Skelton, K., Phifer, J., Jovanovic, T., Gillespie, C.F., Smith, A., Umpierrez, G., Bradley, B., Ressler, K.J., 2011. Posttraumatic stress disorder is a risk factor for metabolic syndrome in an impoverished urban population. *Gen. Hosp. Psychiatry* 33, 135-142.
- Widerlov, E., Lindstrom, L.H., Wahlestedt, C., Ekman, R., 1988. Neuropeptide Y and peptide YY as possible cerebrospinal fluid markers for major depression and schizophrenia, respectively. *J. Psychiatr. Res.* 22, 69-79.
- Wild, S., Roglic, G., Green, A., Sicree, R., King, H., 2004. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* 27, 1047-1053.
- Willis, T., 1675. *Pharmaceutice rationalis sive diabtriba de medicamentorum operantionibus in humano corpore*. In. MDCLXXV, Oxford.
- Wise, R.A., 2008. Dopamine and reward: the anhedonia hypothesis 30 years on. *Neurotox. Res.* 14, 169-183.

- Xu, Y., Berglund, E.D., Sohn, J.W., Holland, W.L., Chuang, J.C., Fukuda, M., Rossi, J., Williams, K.W., Jones, J.E., Zigman, J.M., Lowell, B.B., Scherer, P.E., Elmquist, J.K., 2010. 5-HT(2C)Rs expressed by pro-opiomelanocortin neurons regulate insulin sensitivity in liver. *Nat. Neurosci.* 13, 1457-1459.
- Yamamoto, T., 2007. Brain regions responsible for the expression of conditioned taste aversion in rats. *Chem. Senses* 32, 105-109.
- Yao, Y.J., Geng, D.F., Wang, J.F., Yang, M.H., Zhang, Y.L., Nie, R.Q., Zhou, S.X., 2009. [PPAR gamma agonist rosiglitazone alleviates hypoxia/reoxygenation-induced oxidative stress and apoptosis in rat cardiac myocytes]. *Nan. Fang Yi. Ke. Da. Xue. Xue. Bao.* 29, 689-693.
- Yasojima, K., McGeer, E.G., McGeer, P.L., 2001. Relationship between beta amyloid peptide generating molecules and neprilysin in Alzheimer disease and normal brain. *Brain Res.* 919, 115-121.
- Yasuhara, A., Chaki, S., 2010. Metabotropic glutamate receptors: potential drug targets for psychiatric disorders. *Open. Med. Chem. J.* 4, 20-36.
- Zhong, P., Liu, W., Gu, Z., Yan, Z., 2008. Serotonin facilitates long-term depression induction in prefrontal cortex via p38 MAPK/Rab5-mediated enhancement of AMPA receptor internalization. *J. Physiol* 586, 4465-4479.
- Zhou, L., Sutton, G.M., Rochford, J.J., Semple, R.K., Lam, D.D., Oksanen, L.J., Thornton-Jones, Z.D., Clifton, P.G., Yueh, C.Y., Evans, M.L., McCrimmon, R.J., Elmquist, J.K., Butler, A.A., Heisler, L.K., 2007. Serotonin 2C receptor agonists improve type 2 diabetes via melanocortin-4 receptor signaling pathways. *Cell Metab* 6, 398-405.
- Zraika, S., Hull, R.L., Udayasankar, J., Clark, A., Utzschneider, K.M., Tong, J., Gerchman, F., Kahn, S.E., 2007. Identification of the amyloid-degrading enzyme neprilysin in mouse islets and potential role in islet amyloidogenesis. *Diabetes* 56, 304-310.
- Zupancic, M.L., Mahajan, A., 2011. Leptin as a neuroactive agent. *Psychosom. Med.* 73, 407-414.